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ISBN 0-315-55600-5

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THE UNIVERSITY OF ALBERTA

COMPETITION REACTIONS IN OZONE DISINFECTION

by

SHAHID F. CHAUDHRY

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ENVIRONMENTAL ENGINEERING

DEPARTMENT OF CIVIL ENGINEERING

EDMONTON, ALBERTA
FALL 1989

THE UNIVERSITY OF ALBERTA

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ABSTRACT

This study includes the results of the investigations of the effects of the variations of the system pH and initial bacterial densities on the dose-response relationship in ozone disinfection process. Two separate, covered and uncovered, systems were used. The pH of the systems varied from 4 to 9 and initial bacterial densities used were $10^{4.2}$ CFU/dL, $10^{7.2}$ CFU/dL, $10^{9.3}$ CFU/dL and 10^{11} CFU/dL. *E. coli* was the test organism and 0.05 M phosphate buffer was the medium.

It was found that the variations in the pH of the buffer solution and the initial bacterial densities did not impose any adverse effects on the bacterial die-off. In dose-response experiments, the ozone decomposition was observed directly related with the pH of the test medium. It was also found that the ozone decomposition followed a first order process in this pH range. The total ozone consumption was found higher in uncovered systems and also at higher pH values. The effects of stripping, however, on the total consumption were not significant.

The maximum ozone utilization was found during the first few seconds of the disinfection process and, thus, maximum kill was observed during this stage. The amount of ozone utilized, in terms of number of ozone molecules, to kill one bacterium was calculated and it remained unchanged regardless of the system pH. This quantity, however, was found inversely related with the initial bacterial density. At an initial bacterial density of $10^{4.2}$ CFU/dL, the ozone

molecules utilized were 6 x 10^{11}/bacterium and by increasing the bacterial density to 10^{11} CFU/dL, the number of molecules dropped to 2 x 10^{7}/bacterium. The amounts of OH° radicals produced in the disinfection system, as a result of ozone decomposition, were also calculated. The preliminary calculations showed that OH° radicals did not contribute in the killing of the microorganisms.

ACKNOWLEDGEMENT

The author would like to extend his gratitude to Dr. D. W. Smith for his continued emotional and technical support throughout the laboratory investigations and during the preparation of this manuscript. A thankyou is also extended to Dr. Steve Hrudey for discussing the results and suggesting appropriate steps.

The author is also thankful to the Ministry of Education, Government of Pakistan for providing an opportunity and basic funding to complete this program.

Finally, a special thankyou for my family members for their continuous support, patience and encouragement.

TABLE OF CONTENTS

Title **Page**

1. INTRODUCTION 1
   1.1 BACKGROUND 1
   1.2 MODELLING OF THE DOSE-RESPONSE DATA 4
   1.2 SCOPE OF THE STUDY 5
2. LITERATURE REVIEW 10
   2.1 INTRODUCTION 10
   2.2 CELL STRUCTURE 15
   2.3 DISINFECTION MECHANISM OF OZONE 19
   2.4 OZONE DECOMPOSITION KINETICS 24
   2.5 SELECTION BETWEEN UV AND INDIGO METHODS FOR RESIDUAL OZONE DETERMINATION 35
   2.6 ROLE OF HYDROXYL RADICALS IN OZONE DISINFECTION 39
   2.7 KINETICS OF DISINFECTION 42
   2.8 SELECTION OF THE TEST ORGANISM 52

Title **Page**

2.9 SELECTION OF THE GROWTH MEDIUM FOR *E. coli* 53

3. MATERIALS AND METHODS 55

3.1 ANALYTICAL METHODS 55

3.1.1 Phosphate buffer solution 55

3.1.2 Indigo stock solution 57

3.1.3 Indigo reagent I 57

3.1.4 Indigo reagent II 57

3.1.5 Sodium thiosulphate solution 57

3.1.6 Preparation of ozone stock solution 57

3.1.7 Concentration of ozone stock solution 58

3.1.8 Determination of applied ozone dose 59

3.1.9 Residual ozone concentration 60

3.2 BACTERIOLOGICAL METHODS 60

3.2.1 Tryptone Soya Broth (TSB) 60

3.2.2 0.1% Peptone water 60

3.2.3 Growth medium 61

Title **Page**

3.2.4 Incubation of *E. coli* 61

3.2.5 Test organism 61

3.2.6 *E. coli* densities 62

3.2.6.1 $N_o \sim 10^{4.2}$ CFU/dL 62

3.2.6.1 $N_o \sim 10^{7.2}$ CFU/dL 64

3.2.6.1 $N_o \sim 10^{9.3}$ CFU/dL 65

3.2.6.1 $N_o \sim 10^{11}$ CFU/dL 66

3.3 REACTION VESSEL 67

3.4 PROCEDURES 69

3.4.1 Ozone decomposition studies 69

3.4.2 Dose-response studies 70

3.4.2.1 Bacterial die-off in phosphate buffer solution 70

3.4.2.2 Bacterial die-off in phosphate buffer solution in the presence of sodium thiosulphate 71

3.4.2.3 Ozone dose - E. coli kill studies 72

3.5 QUALITY CONTROL 73

Title **Page**

3.5.1 Ozone demand free water and buffer solution 73

3.5.2 Ozone demand free glassware 74

3.5.3 pH adjustments of buffer solutions 74

3.5.4 Calibration of pippettes 74

3.5.5 Control experiments 75

3.5.6 *E. coli* culturing and subculturing 75

3.5.7 Nutrient agar plates streaking 76

3.5.8 Centrifugation and washing of *E. coli* 76

3.5.9 Determination of N_0 and N 76

3.5.10 Randomization 76

3.5.11 Statistical analysis 77

4. RESULTS AND DISCUSSION 79

4.1 EFFECTS OF pH ON OZONE DECOMPOSITION 79

4.2 EFFECTS OF pH ON BACTERIAL SURVIVAL 125

4.3 EFFECTS OF pH ON OZONE DISINFECTION 131

4.4 EFFECTS OF OH^o RADICALS ON OZONE DISINFECTION 143

Title **Page**

4.5 EFFECTS OF INITIAL BACTERIAL DENSITIES ON BACTERIAL DIE-OFF 147

4.6 EFFECTS OF INITIAL BACTERIAL DENSITIES ON BACTERIAL DIE-OFF 154

5. CONCLUSIONS 180

6. RECOMMENDATIONS FOR FUTURE WORK 184

7. REFERENCES 186

8. APPENDICES 208

LIST OF TABLES

Table **Page**

4.1 Comparison of calculated and tabulated t-values 111

4.2 Half-life and observed k values 115

4.3 Ozone half-life as a function of pH, temperature and medium 116

4.4 k values for covered and uncovered reaction systems 124

4.5 Effects of pH variations on bacterial survival (N_0 ~ 10^7.2 CFU/dL) 127

4.6 Effects of pH variations on ozone disinfection 133

4.7 Effects of pH variations on OH^o radicals production in disinfection process 144

4.8(A) Effects of bacterial densities on die-off (covered system) 149

4.8(B) Effects of bacterial densities on die-off (uncovered system) 150

4.9 Effects of initial bacterial densities variations on ozone disinfection process 157

LIST OF FIGURES

Figure		Page
2.1	Schematic diagram of the cell envelope structure of Gram-positive and Gram-negative bacteria	17
2.2	Detailed structural diagram of Gram-negative cell envelope	18
2.3	Some types of survivor curves observed in disinfection	46
3.1	Reaction vessel	68
4.1 (A-1)	Fitting of ozone decomposition data (Covered vessel, pH 4, n=0)	81
4.1 (A-2)	Fitting of ozone decomposition data (Covered vessel, pH 4, n=0.5)	82
4.1 (A-3)	Fitting of ozone decomposition data (Covered vessel, pH 4, n=1.0)	83
4.1 (A-4)	Fitting of ozone decomposition data (Covered vessel, pH 4, n=1.5)	84
4.1 (A-5)	Fitting of ozone decomposition data (Covered vessel, pH 4, n=2.0)	85
4.1 (B-1)	Fitting of ozone decomposition data (Uncovered vessel, pH 4, n=0)	86
4.1 (B-2)	Fitting of ozone decomposition data (Uncovered vessel, pH 4, n=0.5)	87

Figure **Page**

4.1 (B-3) Fitting of ozone decomposition data (Uncovered vessel, pH 4, n=1.0) 88

4.1 (B-4) Fitting of ozone decomposition data (Uncovered vessel, pH 4, n=1.5) 89

4.1 (B-5) Fitting of ozone decomposition data (Uncovered vessel, pH 4, n=2.0) 90

4.2 (A-1) Fitting of ozone decomposition data (Covered vessel, pH 6.9, n=0) 91

4.2 (A-2) Fitting of ozone decomposition data (Covered vessel, pH 6.9, n=0.5) 92

4.2 (A-3) Fitting of ozone decomposition data (Covered vessel, pH 6.9, n=1.0) 93

4.2 (A-4) Fitting of ozone decomposition data (Covered vessel, pH 6.9, n=1.5) 94

4.2 (A-5) Fitting of ozone decomposition data (Covered vessel, pH 6.9, n=2.0) 95

4.2 (B-1) Fitting of ozone decomposition data (Uncovered vessel, pH 6.9, n=0) 96

4.2 (B-2) Fitting of ozone decomposition data (Uncovered vessel, pH 46.9 n=0.5) 97

4.2 (B-3) Fitting of ozone decomposition data (Uncovered vessel, pH 6.9, n=1.0) 98

Figure		Page
4.2 (B-4)	Fitting of ozone decomposition data (Uncovered vessel, pH 6.9, n=1.5)	99
4.2 (B-5)	Fitting of ozone decomposition data (Uncovered vessel, pH 6.9, n=2.0)	100
4.3 (A-1)	Fitting of ozone decomposition data (Covered vessel, pH 9, n=0)	101
4.3 (A-2)	Fitting of ozone decomposition data (Covered vessel, pH 9, n=0.5)	102
4.3 (A-3)	Fitting of ozone decomposition data (Covered vessel, pH 9, n=1.0)	103
4.3 (A-4)	Fitting of ozone decomposition data (Covered vessel, pH 9, n=1.5)	104
4.3 (A-5)	Fitting of ozone decomposition data (Covered vessel, pH 9, n=2.0)	105
4.3 (B-1)	Fitting of ozone decomposition data (Uncovered vessel, pH 9, n=0)	106
4.3 (B-2)	Fitting of ozone decomposition data (Uncovered vessel, pH 9, n=0.5)	107
4.3 (B-3)	Fitting of ozone decomposition data (Uncovered vessel, pH 9, n=1.0)	108
4.3 (B-4)	Fitting of ozone decomposition data (Uncovered vessel, pH 9, n=1.5)	109

Figure		**Page**
4.3 (B-5)	Fitting of ozone decomposition data (Uncovered vessel, pH 9, n=2.0)	110
4.4	Fitting of ozone decomposition data (pH 4, n=1)	112
4.5	Fitting of ozone decomposition data (pH 6.9, n=1)	113
4.6	Fitting of ozone decomposition data (pH 9, n=1)	114
4.7	Observed first order rate constants of ozone decomposition as a function of [OH^-] ions concentration	121
4.8	Log k vs log [OH^-] ions concentration	123
4.9	Bacterial survival in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, pH 4)	128
4.10	Bacterial survival in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, pH 6.9)	129
4.11	Bacterial survival in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, pH 9)	130
4.12	Ozone consumption in 0.05 M phosphate buffer solution containing E. coli (N_o ~ 10^7.2 CFU/dL, C_o ~ 46 μg/L, pH 4)	134
4.13	Ozone consumption in 0.05 M phosphate buffer solution containing E. coli	

Figure **Page**

(N_o ~ 10^7.2 CFU/dL, C_o~ 46 µg/L, pH 6.9) 135

4.14 Ozone consumption in 0.05 M phosphate buffer solution containing E. coli (N_o ~ 10^7.2 CFU/dL, C_o~ 46 µg/L, pH 9) 136

4.15 Dose-response in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, C_o ~ 46 µg/L, pH 4) 137

4.16 Dose-response in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, C_o ~ 46 µg/L, pH 6.9) 138

4.17 Dose-response in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, C_o ~ 46 µg/L, pH 9) 139

4.18 Number of ozone molecules used per bacterium killed for different pH values (N_o ~ log 7.2 CFU/dL) 141

4.19 Effects of time and pH variations on bacterial survival (in terms of OH radicals production) O3 decomposition: OH radicals production = 1:0.5 (N_o ~ 10^7.2 CFU/dL, C_o ~ 46 µg/L) 145

4.20 Bacterial survival in 0.05 M phosphate buffer solution (N_o ~ 10^4.2 CFU/dL, pH 6.9) 150

4.21 Bacterial survival in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, pH 6.9) 151

Figure **Page**

4.22 Bacterial survival in 0.05 M phosphate buffer solution containing sodium thiosulphate (N_o ~ 10^7.2 CFU/dL, pH 6.9) 152

4.23 Bacterial survival in 0.05 M phosphate buffer solution (N_o ~ 10^9.3 CFU/dL, pH 6.9) 153

4.24 Ozone consumption in 0.05 M phosphate buffer solution containing E. coli (N_o ~ 10^4.2 CFU/dL, Co ~ 46 µg/L, pH 6.9) 157

4.25 Ozone consumption in 0.05 M phosphate buffer solution containing E. coli (No ~ 10^7.2 CFU/dL, C_o ~ 46 µg/L, pH 6.9) 158

4.26 Ozone consumption in 0.05 M phosphate buffer solution containing E. coli (N_o ~ 10^9.3 CFU/dL, C_o ~ 46 µg/L, pH 6.9) 159

4.27 Ozone consumption in 0.05 M phosphate buffer solution containing E. coli (N_o ~ 10^11 CFU/dL, C_o ~ 46 µg/L, pH 6.9) 160

4.28 Dose-response in 0.05 M phosphate buffer solution (N_o ~ 10^4.2 CFU/dL, C_o ~ 46 µg/L, pH 6.9) 161

4.29 Dose-response in 0.05 M phosphate buffer solution (N_o ~ 10^7.2 CFU/dL, C_o ~ 46 µg/L, pH 6.9) 162

4.30 Dose-response in 0.05 M phosphate buffer solution (N_o ~ 10^9.3 CFU/dL, C_o ~ 46 µg/L,

Figure **Page**

pH 9) 163

4.31 Dose-response in 0.05 M phosphate buffer solution(N_o ~ 10^11 CFU/dL, C_o ~ 46 µg/L, pH 9) 164

4.32 Average number of ozone molecules used per bacterium removal for different initial _E. coli_ densities at pH 6.9 167

4.33 Average number of ozone molecules vs time for different initial _E. coli_ densities (pH 6.9) 168

4.34 Formation of peptide bond 171

4.35 Plot of residuals vs calculated Y (model 1) 175

4.36 Plot of residuals vs calculated Y (model 2) 176

4.37 Plot of residuals vs calculated Y (model 3) 177

4.38 Plot of residuals vs calculated Y (model 4) 179

4.39 Number of ozone molecules used to kill one bacterium as a function of pH and N_o (in 30 sec) 183

LIST OF APPENDICIES

Appendix		**Page**
1	Preparation of 0.05 M phosphate buffer solution	209
2	Ozone decomposition in 0.05 M phosphate buffer solution at different pH values	211
3	Bacterial die-off counts data at different pH values (N_o ~ 10^7.2 CFU/dL)	217
4	Dose-response counts data at different pH values (N_o ~ 10^7.2 CFU/dL)	231
5	Summary: dose-response at different pH values (N_o ~ 10^7.2 CFU/dL)	243
6	OH^{o} radicals produced in dose-response experiments	255
7	Bacterial die-off counts data at different initial bacterial densities (pH 6.9)	261
8	Dose-response counts data at different initial bacterial densities (pH 6.9)	274
9	Summary: dose-response at different initial bacterial densities (pH 6.9)	288

1. INTRODUCTION

1.1 BACKGROUND:

The early observations to demonstrate disinfection potential of ozone go back to 1873, when Fox used it to kill molds, fungi and bacteria in the water containing organic matter (Venosa, 1975). Legeron (1984) and Rice *et al.* (1981) mentioned that deMeriterus, in 1886, was one of the earliest scientists to show the germicidal properties of ozone and demonstrated that even the diluted ozonated air would effect the sterilization of polluted waters. Since then, a large amount of fundamental as well as applied research has confirmed the findings of these early works. Among the most important works are those of Fetner and Ingols (1956), Ketzenelson *et al.* (1974) and Sproul and Majumdar (1973). From the literature, it is evident that when ozone is introduced for disinfection purposes, it acts as a very effective and powerful disinfectant (Bean,1959; Dickerman,1954; Ferkinhoff,1936; Nebel *et al.*,1972; Powel *et al.*,1952; Zhu, 1989). Such a universal phenomena, which is entirely different than the other disinfectants in practice, is, basically, a result of the nature of the ozone itself.

Ozone, a reactive species of oxygen, having an oxidation potential of +2.07 volts in acidic solution and +1.24 in basic solution (HOCl = +1.49 volts) at 25°C is a strong oxidizing agent (Venosa, 1975). It is more soluble in aqueous solution than oxygen but is much less soluble than chlorine. For its dissolution Henry's law applies so the commonly low partial pressure in the gas phase makes it difficult to obtain more than a few milligrams per litre concentration in water under normal conditions of temperature and pressure. As with other gases, solubility decreases strongly with rising temperature (Horvath *et al.*, 1985; Venosa, 1975).

Ozone has been used for many years for various purposes; oxidation of mineral compounds such as iron and manganese, oxidation of organic compounds, destruction of trihalomethane precursors, for the oxidation of effluent from paper mills, electroplating plants, refinery wastes etc., improvement in the performance of sand filters, and as a tertiary treatment for municipal wastewaters, and for disinfection of drinking water and wastewaters (Richard, 1985; Roustan *et al.*, 1987; Slater *et al.*, 1985).

In pure water, ozone is very unstable having an effective half life measured in minutes. Because of its instability, its effectiveness as a disinfectant and oxidant, depends upon the rate at which it decomposes (Gurol and Singer, 1982). The self decomposition of ozone (Yurteri and Gurol, 1987) is the result of a chain reaction in which hydroxide ions present in water act as initiators (Hoigne and Bader, 1976), and leads to the formation of a variety of radicals and ions in the aqueous medium including ozone (O_3), hydroxyl radicals (OH°), superoxide ions (O_2^{-}), ozonide ions (O_3^{-}), hydroxide ions (OH^{-}), and free oxygen (Peleg, 1976). This decomposition of ozone is a function of alkalinity (Dore *et al.*, 1987; Peleg, 1976; Venosa, 1975), pH and temperature of the system

(Hoigne, 1982; Hoigne and Bader, 1978). However, if the free radicals are scavenged by solutes such as carbonate and bicarbonate ions (i.e. alkalinity) and thus removed from the solution, the rate of ozone decomposition initiated by the hydroxyl radicals can be reduced (Dore *et al.*, 1987; Hoigne, 1982).

Among the decomposition products of ozone, hydroxyl radicals are the main intermediate species with an oxidation potential of 2.8 volts (Rice and Taylor, 1986). They are extremely reactive and non-selective, being able to attack almost any organic substance (Glaze, 1986). Thus one opinion is that OH° radicals are the main reactive species for the oxidation of soluble organics and inactivation of microorganisms in the ozonation systems (Baxendale, 1964; Dahi, 1976). This group further strengthened its claim by proving that ozone is germicidal only in the presence of water, where it is decomposed to produce OH° radicals (Ewell, 1946; Ingram and Haines, 1949). The other opinion is since OH° are very short lived species (10^{-4} to 10^{-5} sec) with low penetration powers (Nebel, 1985), thus, they can react only with the soluble impurities under well mixed conditions and then diminish before reaching the suspended particulate matter e.g. microorganisms. This may be applied even to those systems where the concentrations of the solutes are appreciably smaller than those of the particles (Hoigne, 1975). But OH° radicals, in many systems, may react with the solutes to form secondary intermediates (e.g. organic peroxy radicals) which become significant over time. These intermediate radicals are of low reactivity but relatively longer half-life so may remain in the system until they come into contact with the dispersed particles and thus contribute in the disinfection process. Hence they are very effective in reacting with soluble impurities but ozone, in its molecular form, may be the only species responsible for killing the microorganisms in ozone disinfection system through direct reaction (Hoigne and Bader, 1976; Hoigne and Bader, 1978).

From the foregoing discussion it can be hypothesized that ozone, in a disinfection system, due to its high oxidation-reduction potential, oxidizes the constituent elements of the cell walls before penetrating through it to react with the enzymes, proteins, DNA, RNA etc. (Farooq *et al.*, 1977). At the same time, it decomposes to form intermediate species, such as OH° radicals, which have even higher oxidation potential than the ozone itself, that are believed to participate in the disinfection mechanism too. This decomposition is basically a function of pH, temperature and presence of organic impurities in the system.

1.2 MODELLING OF THE DOSE-RESPONSE DATA

Most of the ozone disinfection models developed to date, in terms of ozone dose - bacterial response, correlate the survival of microorganisms with some combination of applied or total utilized ozone, temperature, BOD, COD, and contact time and do not accommodate the variations in the pH of the system. Further, during ozonation process, a significant amount of applied ozone dose is lost to the atmosphere (Sugimitsu *et al.*, 1989) but has been reported, in literature, as a part of the ozone consumed during the process, which has led toward the misunderstanding of the ozone potential of disinfecting the microorganisms. Thus the available models may misrepresent the ozone used in the disinfection processes and, moreover, cannot be applied for different pH values. Therefore, it was felt necessary to investigate the dose-response relationship with special reference to these two forgotten parameters and to interpret the data in the form of an equation which involves the actual amount of ozone used in killing the microorganism as well as which accommodates pH changes of the system.

1.3 SCOPE OF THE STUDY

The basic purpose of the experiments performed in the laboratory is to get the indepth understanding of the affects of one or more independent variables on the response under controlled environment. The results derived from such studies are useful, for example, in assessing the biocidal efficiency of the disinfectants. However, with actual field conditions, where the water being treated does not contain clean suspensions of pure cultures of organisms, and a variety of microorganisms are present in their wild state (suspended in a medium that contains a variety of other suspended and dissolved materials, some of which may have significant effects on disinfectant's activity), the killing potential of the disinfectant can be altered or even changed. Thus it can be assumed that the disinfection process does not operate in the same way in the field as it does under laboratory conditions. However, some of these conditions can be simulated in the laboratory experiments and thus provide information that would have more relevance to actual practice.

This study was conducted in different parts using standard procedures. Ozone decomposition studies, at various pH levels, were conducted to determine the amount of ozone that disappeared through the auto-decomposition and stripping to the atmosphere to calculate the exact amount of ozone used in disinfection process. The amount of OH° radicals produced from decomposed ozone were also calculated and efforts were made to correlate the variations in the *E. coli* survival with the amount of OH° present in the system.

The ozone decomposition studies and dose-response experiments were conducted at a wide pH range of 4.0 to 9.0 using 0.05 M phosphate buffer solution at room temperature. The mean initial ozone concentration in the decomposition study was approximately 21 mg/L and the mean applied ozone dose in disinfection experiments was approximately 46 µg/L. The initial bacterial density was varied from $10^{4.2}$ CFU/dL to 10^{11} CFU/dL. All the experiments were conducted using both covered and uncovered reaction vessels. These conditions were selected for the following reasons:

1. To determine the stripping of ozone into the atmosphere and further to investigate its effect on dose-response experiments, covered and uncovered reaction vessels were used. The observations from the covered vessels gave the variations in ozone concentrations due to the auto-decomposition; and observations

from the uncovered vessels gave the variations in ozone concentration due to the auto-decomposition and stripping to the atmosphere. The difference of these two observations, otherwise at same conditions, provided the amount of ozone which disappeared through stripping.

2. Phosphate buffer solution was used because to understand the basic reaction kinetics of the disinfection process it was necessary to conduct experiments in a controlled environment so that the variations in the response could be explained in terms of well known system conditions. A number of researchers (Fetner and Ingols, 1956; Finch, 1987; Perrich *et al.*, 1976; Smith and Bodkin, 1944; Wickramanayake and Sproul, 1988) have used phosphate buffer solution for this purpose.

3. The pH of the medium is one of the important factors in the disinfection potential of many chemicals. It is well established that many disinfectants and antimicrobial agents are effective in a certain pH range. For example, when chlorine is added in water, it is hydrolyzed to form HOCl which is further ionized to give hypochlorite ion, OCl^-. The relative distribution of HOCl and OCl^- depends upon the pH of the system. At pH 4, the chlorine is present in the form of 100% HOCl and at pH 11, 100% OCl^- is present in the chlorination system. As the antibacterial capability of the HOCl is 40 to 80 times higher than that of OCl^-, therefore, the pH of the system is very significant in chlorine disinfection (Metcalf and Eddy, 1979). In the case of ClO_2, the disinfection efficiency increases by increasing pH over this range. Because ClO_2 neither dissociates nor disproportionate into other chemical species in this

pH range, some studies (Benarde, 1965; Hannan, 1953) related this phenomena with the change in the sensitivity of the microorganisms.

In contrast, the ozone does not dissociate in water but decomposes at a rate which, apart from other parameters, strongly depends upon the pH of the system. The higher the pH value, higher is the rate of autodecay, so less ozone is available in its molecular form. Since, apparently, microbial kill is due to the action of ozone molecules, the efficiency of the overall process may be affected.

4. The initial bacterial densities of *E. coli* used were $10^{4.2}$ CFU/dL, $10^{7.2}$ CFU/dL, $10^{9.3}$ CFU/dL and 10^{11} CFU/dL. This covered a wide range of density of organism present in different kinds of waters and wastewaters from surface raw drinking water intakes to the untreated sewage. Further the studies with these initial bacterial densities provided a base to compare results with many other studies available in literature.

5. The incubation temperature selected for *E. coli* was 37°C. Standard Methods (APHA 1985) suggests the use of 35°C with these types of experiments. A number of scientists (Engelbrech *et al.*, 1979; Ishizaki *et al.*, 1987; Roth *et al.*, 1972; Sproul *et al.*, 1979), however, have used a temperature of 37°C to incubate *E. coli* in their studies. The major advantage of using 37°C temperature was that only *E. coli* could survive beyond 35°C and thus no unnecessary growth appeared on the plates.

6. In all the dose-response studies available in literature, the scientists varied the ozone dose while keeping the initial bacterial density constant. This reflected only one side of the research and it was possible that the dose-response experiments gave different

kinds of results if the dose was kept constant and initial bacterial densities were changed. Keeping in view these considerations, a constant ozone dose of approximately 46 µg/L was used in all the disinfection experiments and the initial bacterial densities were changed from $10^{4.2}$ CFU/dL to 10^{11} CFU/dL.

7. The very particular reason of selecting some of these conditions was to compare some of the results with the work of Finch (1987).

2. LITERATURE REVIEW

2.1 INTRODUCTION

Disinfection is one of the most important unit processes involved in water treatment. Whether the particular water is to be used for potable purposes, or to be discharged to the environment, the goal of disinfection is to destroy pathogenic organisms and thus prevent the transmission of diseases through water consumption. Ozone is one of those agents applied to achieve this goal.

Traditionally, free chlorine has been the disinfectant of choice which is also a powerful oxidant (Glaze, 1987). When applied, it reacts with the trace amounts of natural organic compounds present in water and forms chloro-organic compounds, like trihalomethanes (THM), which are suspected carcinogens and thus possess a risk to the consumers (Vogt and Regli, 1981). The need for the elimination or reduction of the THM precursors to an acceptable levels has forced water utilities to investigate the appropriate substitutes. Various alternate disinfectants like chloramines, chlorine dioxide and ozone, have been tried (Glaze, 1987; Greenberg, 1981; Hoff and Geldrich, 1981; Vogt and Regli, 1981), and ozone is proven to be the most potent biocide. It is still not confirmed when ozone was first used for disinfection, however, it has been in use for different purposes for over a hundred years (Venosa, 1975). The attractions which make it a disinfectant of preferred choice over other chemicals include formation of much smaller amounts of mutagens than with other disinfectants like ClO_2 and Cl_2, aid in coagulation process and generation of radical intermediates which are stronger oxidizing agents and may have more disinfection power than ozone itself (Glaze, 1987; Greenberg, 1981; Prendiville, 1986; Rice *et al.*, 1981; Rosen, 1976; Zoeteman *et al.*, 1982). The other benefits associated with its use as a disinfectant can be summarized as follow:

1. An excellent virucide as well as bactericide.
2. Shorter treatment times (1 to 10 minutes for ozone compared to 30 to 45 minutes with chlorine).
3. Lesser effects of pH and temperature on disinfection efficiency.
4. As excess ozone decomposes into oxygen with a short half life, oxygen remaining in the water may have the favourable effect of eliminating the necessity for subsequent aeration of water.
5. No toxicity to aquatic life has been observed.
6. No highly refractory or bioaccumulatable residuals have been observed or are predicted to form in ozonated municipal effluents.
7. There is no increase in total dissolved solids in ozone-treated water. This is especially important where effluent reuse is considered.
8. Wastewater quality improvements such as effluent colour and turbidity reductions always accompany ozone disinfection.

9. Ozone treatment may easily be extended as a tertiary polishing step by the addition of more ozone dose and/or by extending the contact times.

At the same time, there are some disadvantages associated with the use of ozone as a disinfectant e.g.

1. Quick decomposition of residual ozone may constitute a set back because no residual ozone can be maintained in the system to prevent the bacterial recontamination.
2. The effect of ozonization is strongly dependent on the water quality. Thus, in the case of a high concentration of organic substances, for example, the efficiency of the ozone markedly decreases. At the same time, the ozone consumption rises because a large portion of the applied ozone is off-targeted by oxidizing extraneous compounds and failing to achieve the intended aim.
3. A further disadvantage is the necessity to generate ozone on site. This feature, however, is some times regarded as an advantage especially in the case of continuous large-scale production.
4. High capital and operational costs.

All of the above benefits are true within the present state of knowledge. The study of ozonation processes, however, has not been as extensive as that of to the chlorination processes and, therefore, it does not mean that ozonation necessarily satisfy all aspects of safety of the treated water. It is quite possible that the situation with ozone treatment may be analogous with the chlorine; where chlorine was applied for many decades for disinfection purposes before the THM were discovered. Similarly there may be by-product formation during ozone treatment which are more toxic than their precursors but have not found yet. The disadvantages associated with the use of ozone can be eliminated or by-passed in the majority of the cases, leaving only the economic factor associated with the large capital outlay requirements and high operational costs as the only essential disadvantages. This is more and more being off set, however, partly by advantages over other methods and partly by the strict purification requirements that can not be met otherwise. In addition to this, if the use of any particular chemical is connected with the health protection and protection of the environment, the technical feasibility to achieve this target with ozone may slowly displace the economic difficulties as time goes on.

Ozone is very selective in reacting with other compounds. These reactions can be classified as very reactive, moderately reactive and slow reactive, according to the reaction rates with which ozone reacts with them. Hoigne (1982) has shown that even different compounds from one class do not follow the same reaction order. Oxidation of phenols, nitrophenols, chlorophenols and cresols falls under the category of very fast reactions with high k values in the range of 10^7/M sec. The oxidation of these compounds is completed within minutes even at low ozone concentrations. Medium fast reactions are those with k = 1 to 10,000/M sec. The practical ozonation processes relevant for these solutes and the rate of the chemical reaction determines the overall reaction rate. Benzene (k=2/M sec), naphthalene (k=3,000/M sec), toluene (k=14/M sec) and methylamine (k=280/M sec) are typical examples of this class. The slow reactions are those, by definition, having k values less than 1/M sec. These reactions are so slow that their life time may exceed 10,000 sec., even at elevated ozone concentrations, which is not feasible for practical oxidation. Examples are ozonation of nitrobenzene (k=0.1/M sec), benzenesulfonate (k=0.2/M sec), ammonia (k=1/M sec) and glucose (k=0.5/M sec).

The reactions of hydroxyl radicals with organic solutes, on the other hand, are extremely rapid, non-selective and faster than the ozone. For example, the reaction rate constants with trichloroethylene and benzene are 2.6×10^9 and 6×10^9/M sec respectively (Glaze *et al.*, 1987, Hoigne, 1982). Thus it can be said that hydroxyl radicals may not take part in the inactivation of microorganisms rather they oxidize the organic compounds. This statement, however, can not be generalized for those systems containing trace amounts of pollutants and high concentrations of bacteria because in such systems, the low concentrations of organic impurities have a low probability of being attacked by the hydroxyl radicals (Glaze *et al.*, 1987). Because in such systems, the hydroxyl radicals have to travel relatively long distances to come across a soluble species and in that case they could easily be encountered by the high densities and bigger size of the suspended particles, and because of their non-selective reaction nature, they can react with the microorganisms, or in other words, disinfection could take place. Thus, it can be postulated that in the systems containing very low concentrations of soluble compounds and high bacterial densities, both hydroxyl radicals and ozone may contribute in killing of the bacteria.

2.2 CELL STRUCTURE

The bacterial cell, basically, consists of a rigid cell wall enclosing the cytoplasm which itself is enclosed in the cytoplasmic membrane (Bailey and Ollis, 1986; Sonnenwirth, 1980). Under favourable conditions, the cell is subject to growth in an orderly manner and its life cycle is accomplished by virtue of its indigenous enzymic constitution (Rose, 1965; Sykes, 1965). These enzymes in a bacterial cell have at least four functions (Gale, 1943; Bailey and Ollis, 1986):

1. to release energy for continued existence and division,
2. to provide essential metabolites and nutrilites,
3. to detoxify toxic metabolic products, and
4. to stabilize the internal environment in a variable external environment.

By these actions the organism is able to select suitable nutrient substances from the surrounding medium and modify them by appropriate synthetic and breakdown processes to make materials for cell structure and for reproduction on a strictly genetic basis, giving rise to many end products including nucleic acids, antigentic substances, polysaccharides, toxins and the like, all of which form the basic characteristics of the cell (Sykes, 1965). Since enzymes are proteins (Bailey and Ollis, 1986), therefore, they are subjected to inactivation through coagulation or denaturation by heat or other physical means, and by a wide range of chemicals (Sykes, 1965).

Considerable research has been done on the molecular organization of the surface layers of the Gram-negative bacterial cells (Braun, 1978). Peptidoglycan is the major component outside of the cytoplasmic membrane. It constitutes a relatively thick fibrous layer interspersed by teichoic and teichuronic acids and lipids. Outside this peptidoglycan layer, the Gram-negative bacteria have an additional layer known as outer membrane which appears similar to the cytoplasmic membrane but is entirely different in biochemical composition (see figure 2.1). It contains less phospholipids, fewer types of proteins and lipopolysaccharides (LPS).

These LPS are entirely located at the outer surface of the the outer membrane while the phospholipids are present at the inner face of this membrane (Kamio and Nikaido, 1976). Verkleij *et al.* (1977) have mentioned that there are four or five major proteins present and are exposed at the outer surface. These proteins are arranged in such a way as to make water-filled channels known as 'porins'. Osborn and Wu (1980) have indicated the location of these compounds in the outer layer as shown in Figure 2.2. De Rienzo *et al.* (1978) have mentioned that these porins are produced by three molecules of proteins, each held in place by the triple coiled structure of a special lipoprotein molecule, which passes through the outer membrane and is covalently bonded with the underlying peptidoglycan. These pores are believed to be 1.5 to 2.0 nm in

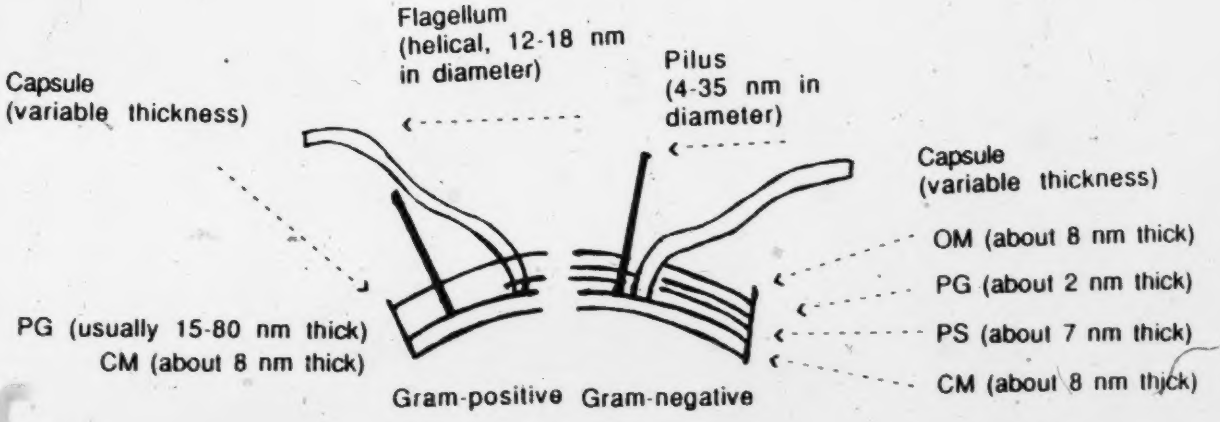

Capsule
(variable thickness)
Flagellum
(helical, 12-18 nm
in diameter)
Pilus
(4-35 nm in
diameter)
Capsule
(variable thickness)
OM (about 8 nm thick)
PG (about 2 nm thick)
PS (about 7 nm thick)
CM (about 8 nm thick)
PG (usually 15-80 nm thick)
CM (about 8 nm thick)
Gram-positive
Gram-negative

Fig. 2.1 Schematic diagram of the cell envelope structure of Gram-positive and Gram-negative bacteria (CM: cytoplasmic membrane, PG: peptidoglycan, PS: periplasmic space, OM: outer membrane)
(adapted from Bailey and Ollis, 1986)

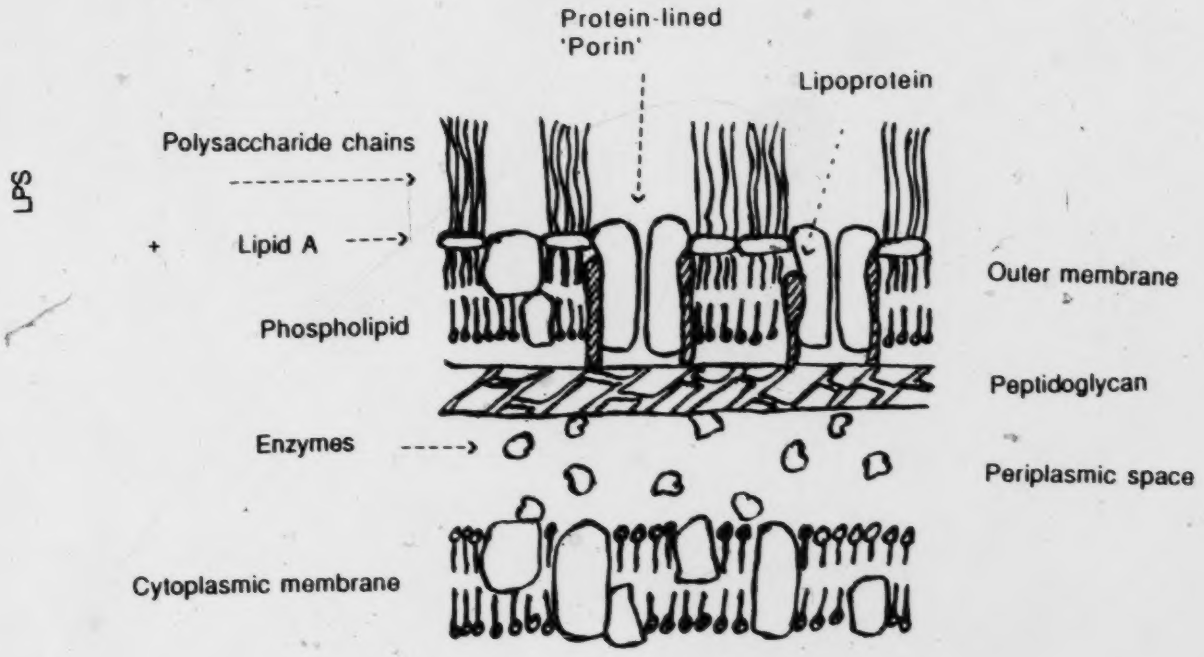

Protein-lined
'Porin'
Lipoprotein
Polysaccharide chains
LPS
+
Lipid A
Phospholipid
Outer membrane
Peptidoglycan
Enzymes
Periplasmic space
Cytoplasmic membrane

Fig. 2.2 Detailed structural diagram of Gram-negative cell envelope (adapted from Osborn and Wu, 1980)

diameter and allow the passage of low molecular weight nutrients such as amino acids, sugars, salts, etc. into the periplasmic region. Synthesis of these nutrients takes place in the periplasmic region and the required products are transported into the cytoplasm. Decad and Ninkaido (1976) have indicated that these porins impose a sharp exclusion limit in terms of molecular size and "the outer membrane of *E. coli* appears to constitute a permeability barrier for hydrophilic compounds with molecular weight greater than 550 to 600".

2.3 DISINFECTION MECHANISM OF OZONE

A number of possible disinfection mechanisms with different types of disinfectants have been postulated. Generally, it is believed that germicides and sterilizing agents interfere with the metabolism of bacterial cells, most likely through inhibiting and blocking the operation of the enzymatic control system. A sufficient amount of oxidizing agent breaks through the cell membrane and this leads to the destruction of bacteria (Horvath *et al.*, 1985). Kulikovsky *et al.* (1975) observed that the damage to the cell membrane and inhibition of biochemical activities associated with this are responsible for bacterial inactivation. Inhibition of specific enzymes and enzyme systems have also been suggested as inactivating mechanisms. Sykes (1965) mentioned that the lethal action of a disinfectant is due to its capacity to react with the protein and, in particular, to the essential enzymes of the microorganism. Therefore, any treatment which inactivates one or more essential enzymes of the cell, or which so affects an essential metabolite that it is rendered unavailable to the enzyme, produces, in effect, a break in the life cycle of the cell with the result that the cell is unable to reproduce and so, by definition, is presumed ineffective. Keswick *et al.* (1981) and O'Brien and Newman (1979) have postulated that, in viruses, both nucleic acids and surface proteins are sites for halogen disinfectants activity.

Thus the manner in which chemical disinfectants exert their germicidal activities varies with the type of the compound and can be categorized as (Sykes, 1965):

1. adsorption and reaction on the cell wall,
2. penetration and reaction with the constituents of the cell protoplasm, and
3. reaction of the compound with one or more of the cell constituents.

Like many other aspects of ozone disinfection, there is a wide difference of opinions on how ozone inactivates the microorganisms. In 1954, Giese and Christensen (1954) exploited the ozone potential to bleach various pigments to observe its action on the organisms. To observe if the ozone enters a given cell, they stained the bacteria with aqueous neutral red and placed in droplets over ozonized water. Observations indicated that the colour of the stained bacteria remained unchanged as long as the bacteria were intact. Once they were badly injured by ozone, as indicated by vesciculation, they were quickly decolourized. This suggested that ozone did not at once penetrate into the cell membrane but produced its initial effects only on the surface of the cell. Only when the cell membrane had been injured, did it enter. Thus the primary activity of ozone was on the bacterial cell surface.

Barron (1954) predicted that in system of biological importance, the oxidation of sulfhydryl (S-H) compounds by radicals, like OH°, O_2H and H_2O_2, is the most promising step towards the deactivation of microorganisms. As non-protein -SH compounds regulate the mechanisms of cell metabolism and are essential for cellular division as well as for cellular growth; their oxidation to disulfides (S-S) will interrupt this process and, in other words, will inactivate the cell.

Scot and Lesher (1963), on the other hand, determined that the (S-H) concentration of the bacteria was not decreased until it leaked out or the cell was lysed. Hence, they postulated that the primary attack of ozone was on the cell wall or the membrane of the bacteria, probably by reaction with the double bonds of unsaturated lipids.

Murray *et al.* (1965) recognized that the outer most layer of the Gram-negative organisms is a lipoprotein followed by lipopolysaccharides layer. In case of ozone disinfection, these layers are attacked first by ozone, cell permeability is changed which eventually leads to the cell lysis. Smith (1969) stated that ozone oxidized the unsaturated fatty acids (mainly C16 and C18 monoenoic acids) of the cell lipids; and because lipids present in bacteria are mainly confined to the cytoplasmic membrane, so Smith (1969) agreed with Christensen and Giese (1954), Giese and Christensen (1954) and Scot and Lesher (1963).

Perrich *et al.* (1975, 1976) concluded from their studies that the cell lysis was not the primary mechanism of ozone inactivation of *E. coli.* They did not find any specific mechanism but speculated a few alternatives as:

1. Cell membrane disruption so that critical cellular components diffuse out of the cell, or
2. Irreversible inhibition of enzymes or alteration of other critical components of the cellular cytoplasm, or
3. Inactivation of the nucleic acids.

Pryor *et al.* (1983) suggested that the inactivation mechanism of bacteria with ozone should be more complicated because ozone attacks proteins and unsaturated lipids of the cell membrane and also enzymes in the cells. Ishizaki *et al.* (1987) postulated that ozone penetrates cell membrane and reacts with cytoplasmic substances and, therefore, chromosomal deoxyribo-nucleic acids (DNA) might be one of the targets of the ozone degradation, and its damage might be one of the factors responsible for the cell death.

In a recent study, Mehlman and Borek (1987) concluded from other researchers findings that the action mechanisms of ozone on bacteria can be divided into two groups as follow:

1. The ozone acts by initiating peroxidation of polyunsaturated fatty acids present mainly in cell membrane. The other secondary decomposition products produce their toxicity by

damaging the integrity of the cell membrane and other cellular molecules, and

2. Ozone exerts its toxicity by oxidation of low molecular weight compounds (e.g. amine, aldehyde and alcohol functional groups containing compounds) and by oxidation of proteins.

Both soluble peptides and proteins in lipids provide targets for ozone disinfection. These two mechanisms may be interrelated, because peroxidation of poly unsaturated fatty acids give rise to water-soluble products such as aldehydes, peroxides and OH° radicals (Pryor *et al.*, 1983) which diffuse into the cytosol and initiate oxidation of amino acids and proteins (Borek and Mehlman, 1983).

The direct oxidation of amino acids and proteins by ozone or oxidation by secondary reaction products of poly unsaturated fatty acids can inhibit a variety of cellular protective systems. The degree to which ozone reacts with proteins is determined by the presence of ozone susceptible amino acids at their active sites and the location of amino acids in the proteins (Freeman and Mudd, 1981; Mudd and Freeman,1977). Because the maintenance of the structure of membrane protein is dependent upon the associated lipids, any alterations in lipids surrounding the protein results in structural alterations and changes in membrane functions.

At the same time, ozone exposure to the enzymes convert thiols into disulfides. Thiols are an essential part of the active site of many enzymes; thus oxidation of these active site thiols to disulfides inactivates the enzymes. Thus it is possible that both mechanisms prevail upon ozone exposure, i.e.

- Direct oxidation of the low molecular weight compounds and proteins, and
- Lipids peroxidation with its subsequent chain reaction.

This discussion explains that the exact inactivation process of the cells and the contribution of the penetration through the cell membrane and the part played by the various reactions taking place in metabolism are not yet fully understood. Moreover, since ozone is a powerful oxidizing agents, it is very likely that the effect may be on the many vital functions making it very difficult to determine a specific site or function on which it exerts it lethal effects. Thus more intensive research is needed before any specific disinfection mechanism of ozone disinfection could be established.

2.4 OZONE DECOMPOSITION KINETICS

Ozone kinetic studies were ancillary in many of the early investigations (Weiss, 1935). From these studies, however, it is believed that many observations about ozone decomposition were noted and gradually it became an area of active investigation.

Roth and Sullivan (1983) and Sugimitsu *et al.* (1989) have indicated that Rothmund and Burgsteller, in 1913, were, probably, the first ones to suggest the following empirical model of ozone decomposition based on their studies at 0°C:

$$-dO_3/dt = k_1(a - O_3) + k_2(a - O_3)^2$$

where a, k_1 and k_2 are determined experimentally.

According to their studies, the decomposition in acidic solution was of second order and so the second term in the equation dominated in acidic solutions; while in weaker acidic and alkaline solutions, the decomposition was of first order. These investigators reported the lack of reproducibility of results in acidic medium which was later attributed to small but variable amounts of hydrogen peroxide which catalytically decomposed the ozone in solution. Sennewald (1933) found that the order of ozone decomposition was consistent for various buffer solutions at a constant pH but the specific rate constant was the function of the reaction system. He correlated the ozone decomposition with the pH of the buffer of the solution and determined a 0.36 order for the OH^- ion concentration.

Since then many workers have attempted to study the decomposition of ozone in aqueous solutions using different decomposition reactions and under different working conditions like ozone concentrations, pH, ionic strength, buffer present or absent, possible scavengers and promoters present, etc. The observed decomposition kinetic orders have ranged from 0.5 to 2.0.

Hoigne and Bader (1975) have proposed that when ozone is added in demand free water, it decomposes by its reaction with OH^- ions present in equilibrium with water:

$$H_2O \leftrightarrow H^+ + OH^- \qquad pK_w = 14.17 \text{ at } 25^\circ C$$

Because the initiation reaction is pH dependent, the rate of decomposition is a function of pH of the water. There are different proposed mechanisms for this reaction. Weiss (1935) hypothesized the following decomposition path:

$$O_3 + OH^- \rightarrow O_2^{o-} + HO_2^{o}$$

$$HO_2^{o} \leftrightarrow O_2^{o-} + H^{o} \qquad (pK_a = 4.8)$$

$$O_3 + O_2^{o-} \rightarrow O_2 + O_3^{o-}$$

$$O_3^{o-} + H_2O \rightarrow OH^{o} + OH^- + O_2$$

He derived the following model for the ozone decomposition in terms of pH and ozone concentration of the system:

$$-dO_3/dt = k_1 \, [O_3] \, [OH^-] + k_2 \, [O_3]^{1.5} \, [OH^-]^{0.5}$$

In acid region, he observed that decomposition kinetic was of 3/2 order while at high pH values, where hydroxide ions were in significantly high concentrations, the rate followed first order.

Temperature is another factor which expedites the dissociation of water; therefore, higher the temperature is, lesser would be the molecular ozone available in the solution. Ewell (1941) determined that the action of ozone was greater at low temperature because of its greater solubility and slower decomposition rate than at higher temperatures.

Alder and Hill (1950) proposed the following path for ozone decomposition:

$$O_3 + H_2O \rightarrow HO_3^+ + OH^- \qquad \text{(reaction rate constant, } k_1\text{)}$$

$$HO_3^+ + OH^- \leftrightarrow 2HO_2 \qquad \text{(reaction rate constants, } k_2 \text{ \& } k_2'\text{)}$$

$O_3 + HO_2 \rightarrow HO + 2O_2$ (reaction rate constant, k_3)

$HO + HO_2 \rightarrow H_2O + O_2$ (reaction rate constant, k_4)

The overall reaction was:

$$2O_3 + H_2O \rightarrow H_2 + 3O_2$$

They (Alder and Hill, 1950) investigated the ozone decomposition by varying the pH of the aqueous solutions (0.1 and 0.2 N solutions of perchloric acid) from 1.01 to 2.73 at two different temperatures, 0°C and 27°C. Based on their data, they modeled, satisfactorily, first order decomposition rate with respect to ozone concentration. To accommodate the effects of temperature and pH on the decomposition rate, K, the equilibrium constant, and OH^- terms, were incorporated, respectively, in the model which was:

$$d\{O_3\}/dt = -3k_3 K^{1/2} \{HO_3^+\}^{1/2} \{OH^-\}^{1/2} \{O_3\}$$

The integral form of this model was:

$$d\{O_3\}/ \{O_3\}_o = -3k_3 K^{1/2} \{HO_3^+\}^{1/2} \{OH^-\}^{1/2} .t$$

Similarly, Stumm (1954) studied the reaction rate over pH range of 7.6 to 10.4 at three different temperatures 1.2°C, 14.6°C and 19.8°C and correlated the data using first order rate expression with respect to dissolved ozone. However, he observed that the ozone decomposition rate increased due to the presence of trace amounts of both inorganic and organic compounds as well as by increasing the pH of the system. He described his model, in water, as:

$$\log\{O_3\}_o/\{O_3\} = k \{OH^-\}^{0.75} t$$

At constant temperature of 14.6°C, the half-life of ozone was 10.5 min at pH 8.5 and it reduced to 1 min. at pH 10.4. Similarly, he determined the dependence of k on temperature was exponential. This supported the work of Alder and Hill (1950) who based on their proposed decomposition mechanism of ozone in aqueous solutions, included K, the equilibrium constant, in their model.

Kilpatric *et al.* (1955) found that the ozone decomposition order was 3/2 in acidic (0.01 M $HClO_4$) and in phosphate and arsenate buffered solutions. In alkaline (NaOH) solution, the rate was proportional to the second order with respect to ozone concentration.

In recent years, Sullivan and Roth (1980) modeled the ozone decomposition data obtained over a wide range of pH and temperature. A multilinear regression analysis was performed to establish the dependence of specific reaction rate constant on the pH and temperature of the solution, which turned out as:

$$k = 9.811 \times 10^7 \exp [(-5606/T) (OH^-)^{0.123}]$$

The over all rate equation for ozone decomposition was first order with respect to ozone and was described as:

$$-d[O_3]/dt = 9.811 \times 10^7 \exp [(-5606/T) (OH^-)^{0.123} [O_3]]$$

Joy *et al.* (1980) and Staehelln and Hoigne (1982) were in favour of a less complicated ozone decomposition route as:

$$O_3 + OH^- \rightarrow HO_2^- + O_2$$

$$O_3 + HO_2^- \rightarrow OH^o + O_2^{o-} + O_2$$

Gurol and Singer (1982) studied the kinetics of ozone decomposition in a batch reactor under steady state conditions, and confirmed under dynamic conditions, that it could be approximated by a second order reaction in the pH range of 2 to 9.5, and expressed as:

$$r = -d[O_3]/dt = k_o\ [OH^-]^{0.55}\ [O_3]^2$$

Tomiyasu *et al.* (1985) observed the decomposition of ozone in a basic solution ($[OH^-] = 0.007$ M) involving first and second order terms with respect to ozone. The decomposition kinetics satisfied the following empirical equation:

$$-d[O_3]/dt = k_1\ [O_3]\ +\ k_2\ [O_3]^2$$

The values of k_1 and k_2 were strongly affected by the solution composition. Unlike other equations, the hydroxyl ion concentration was not included in this relationship, but Tomiyasu *et al.* (1985) observed that the equation was dependent on the system pH and at higher concentrations of OH^- ions, the rate law changed significantly. The second term on the right hand of the equation was dependent on the presence of radical scavengers like Na_2CO_3 and with the increasing concentration of Na_2CO_3, this term disappeared gradually and the rate equation became first order eventually.

Gordon and Pacey (1986) proposed the following mechanistic reactions for ozone decomposition:

$$O_3 + OH^- \rightarrow HO_2^- + O_2$$

$$O_3 + HO_2^- \rightarrow O_3^- + HO_2$$

$$HO_2 + OH^- \rightarrow O_2^- + H_2O$$

$$O_2^- + O_3 \rightarrow O_3^- + O_2$$

$$O_3^{\cdot} + H_2O \rightarrow OH^{o} + O_2 + OH^{\cdot}$$

The overall decomposition reaction in this case can be cited as:

$$3O_3 + OH^{\cdot} \rightarrow O_3^{\cdot} + OH^{o} + 3O_2$$

Based on their decomposition equations, Gordon and Pacey (1986) suggested the following empirical model for the decomposition of ozone in aqueous medium which was first order with respect to $OH^{\cdot}$ and second order with respect to O_3:

$$r = -d[O_3]/dt = k_{OH^{\cdot}} [O_3][OH^{\cdot}] + k_2 [O_3]^2[OH^{\cdot}]$$

The values of $k_{OH^{\cdot}}$ and k_2 were dependent on the solution composition (e.g. pH, ozone concentration and presence of scavengers). Sugimitsu *et al.* (1989) studied the decomposition of ozone in water over a pH range of 1 to 9 and temperature of 1°C to 30°C. They found that stripping was main cause of ozone concentration reduction from the water. Preventing such escape of ozone from the liquid, they determined a very low reaction rate constant of 5.22×10^{-6}/sec at 25°C for pH < 4. At higher pH values, the k became a function of pH as:

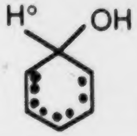
$$k^{*} = k\,[OH^{\cdot}]^{0.7}$$

where k was temperature dependent.

In the presence of organic trace impurities in water, the self decomposition of ozone takes place in the same way as in pure water and leads to the formation of hydroxyl radicals (Hoigne and Bader, 1976; Staehelln and Hoigne, 1985). All organic and inorganic constituents of the liquid phase compete for hydroxyl and other radical species, and thus indirect, second order, oxidation with hydroxyl radicals takes place (Hoigne and Bader, 1976). The indirect oxidation of impurities follow three different ways (Hoigne, 1982):

1. Radical addition reaction:

OH^{o} + benzene →



(k = 6 x 10^{9}/M sec)

In contrast, the reaction of benzene with ozone in water is very slow, k = 2/M sec, and takes many hours to complete at elevated temperatures (Nebel, 1985).

2. Hydrogen abstraction reaction:

$$OH^{o} + CH_3CH_2OH \rightarrow H_2 + CH_3CHO^{o}H \qquad (k = 2 \times 10^{9}/M\ sec)$$

3. Electron transfer reaction:

$$OH^{o} + CO_3^{--} \rightarrow OH^{-} + CO_3^{o-} \qquad (k = 3 \times 10^{8}/M\ sec)$$

The secondary radicals (R^{o}) formed by these reactions may react with each other. However, in an ozonated system there is always an ample amount of dissolved oxygen so that the radicals may add an oxygen molecule before they encounter each other (Hoigne, 1982) or they may react with the environmental molecular oxygen (Uri, 1953) to form an organic peroxy radical:

$$R^{o} + O_2 \rightarrow ROO^{o} \qquad \text{(equ. A)}$$

The peroxy radicals formed in this manner act as strong oxidants and can abstract hydrogen atoms from many types of organic compounds ($R'H$):

$$ROO^o + R'H \rightarrow R'^o + ROOH$$

A new radical, R'^o, is formed which again may add oxygen. In principle, chain reactions that may lead to the autooxidation may become operative. In natural waters, however, such chain reactions are quenched by many types of dissolved impurities which act as chain terminating reagents. The hydroperoxides might also decompose as follows:

$$2\,ROOH \rightarrow H_2O + RO^o + ROO^o$$

$$ROOH \rightarrow RO^o + OH^o$$

The chain terminating reactions could be due to the mutual reactions of the radicals if there concentrations become high (Hewes and Davison, 1971) and may lead as:

$$2\,ROO^o \rightarrow \text{products}$$

$$ROO^o + R'^o \rightarrow \text{products}$$

$$2\,R'^o \rightarrow \text{products}$$

When dissolved organic compounds are oxidized, carbon dioxide, a product of oxidation, increases in the aqueous system to form H_2CO_3 and in turn ionizes to produce bicarbonate and carbonate ions:

$$CO_2 + H_2O \rightarrow H_2CO_3$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad (pK_a = 6.3 \text{ at } 25^\circ C)$$

$$HCO_3^- \leftrightarrow H^+ + CO_3^{--} \qquad (pK_a = 10.3 \text{ at } 25^\circ C)$$

Both of these anions are very effective in terminating the free radical reactions. Thus as the oxidation of organic compounds progresses, there is an increased force produced to stop the oxidation by elimination of hydroxyl radicals. Carbonic acid is not a free radical trap, therefore in the oxidation systems, the pH is kept low so that predominant form of the dissolved carbon dioxide is the undissociated carbonic acid (Nebel, 1985). This supports Hoigne and Bader's (1976) work that the reaction rate constant of hydroxyl radicals with carbonate ions (k = 20 x 10^7/M sec) is higher than with bicarbonate ions (k = 1.5 x 10^7/M sec), and thus the scavenging effect is due more to carbonate ions. They also showed that the reaction rate constant for reactions of hydroxyl radicals with organic molecules was fairly insensitive to the variations in parameters such as pH and salt concentrations.

The above literature review on ozone decomposition reveals that a wide range of decomposition theories and models have been developed but they satisfy only the very specific conditions under which they were formulated and many discrepancies exist both in the reaction order of the ozone decomposition kinetics as well as in the associated reaction rate constant (Peleg, 1976), which has been termed as "system specific rate constant" by Tomiyasu *et al.* (1985). Even work performed over common ranges of pH and temperature has resulted in different conclusions. Gurol and Singer (1982) mentioned that these variations in decomposition order were due to:

1. The use of different, some times questionable, analytical techniques to measure the concentration of dissolved ozone,

2. Uncertainties in the data analysis and data interpretation,
3. The effect of solution decomposition, e.g. the ionic medium of decomposition, and
4. The possible presence of impurities in the reagents used.

Researchers generally agree that the main parameters which affect the ozone decomposition rate are temperature, pH and the presence or absence of impurities. Hoigne and Bader (1979b) have shown that in a typical, natural, clean water at about pH 8, about half of the ozone introduced is decomposed within about 10 minutes. Grunwell *et al.* (1983) showed ozone half life as 8 minutes at pH 7.0 in purified water. The initial concentration of ozone in water was about 10 mg/L. Watson (1944) found that action of ozone was about the same between 4.30 and 7.95 but increased below pH 4.3 on *E. coli*. Gordon and Pacey (1986) mentioned that the change in pH, the presence of catalytic intermediates ($HO_2^{\cdot}$, $O_3^{\cdot}$, $O_2^{\cdot}$, OH^{o}), and the high concentration of dissolved oxygen affects the speciation of ozone itself. Among all the species resulted from ozone decomposition hydroxyl radical (OH^{o}) is the dominant. This free radical does not carry any +ive or -ive charge, so is a neutral species. It has an unpaired electron and, therefore, is denoted with a dot on the oxygen atom (Nebel, 1985). It is a very reactive, non-selective and very short lived species which has low penetrating power, therefore, a good mixing is required in the system where this radical is to be used.

2.5 SELECTION BETWEEN UV AND INDIGO METHODS FOR RESIDUAL OZONE DETERMINATION

The analytical methods applied for the determination of residual ozone concentration can generally be grouped as iodometric and non-iodometric methods (Grunwell *et al.*, 1986). Different versions of the iodometric procedures basically count on the oxidation of iodide to iodine by ozone according to the following equation:

$$O_3 + 2\,I^- + H_2O \rightarrow I_2 + 2\,OH^- + O_2$$

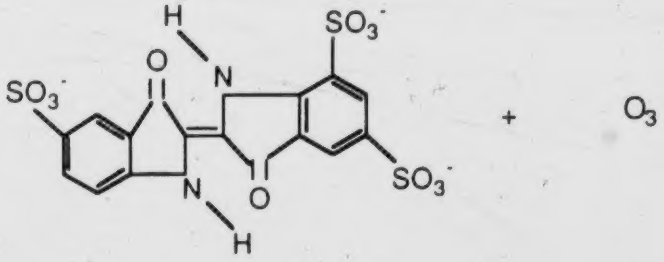
Due to the relative ease of oxidation of iodide and reduction of iodine, these methods are highly susceptible to interferences from other oxidizing and reducing agents (Stanley and Johnson, 1986) and may respond even in the absence of ozone (Gurol, 1980). Gordon and Pacey (1988) and Gordon and Grunwell (1984) are of the opinion that iodometric techniques to determine residual ozone concentration are "non-selective" and the use of these methods is "unacceptable" in water treatment.

Among the non-iodometric methods, UV spectrophotometric measurements and indigo determination are the methods of choice for aqueous ozone determination. UV measurement is successfully applied for molecular ozone determination both in the air and water but has certain limitations. In air, ozone shows a range of strong absorption bands between 200 to 300 nm, known as Hartley bands, with an absorption peak at 253.7 nm (Horvath *et al.*, 1985). The determination of the molar absorptivity is based on the measurement of the optical absorption of monochromatic light at this wavelength in a gas absorption cell with quartz glass windows. In literature the values of molar absorptivity varies from 2,999 to 3,600 L/mole-cm, and the biggest difficulty lies in the selection of the exact value (Maier and Kurzmann, 1986). This wide range of values is a result of all component of the gas mixture which absorb UV light at 253.7 nm wavelength. Under laboratory conditions, however, where precleaned air or oxygen is used to produce ozone, these interferences can be neglected. EPA and European Standardization Committee of the International Ozone Association (IOA, 1987) recommend the use of 3,000 L/mole-cm, and 3,000 ± 30 L/mole-cm, respectively, at 253.7 nm (at the mercury resonance) to determine the ozone concentration in a process gas and in the atmosphere by UV absorption.

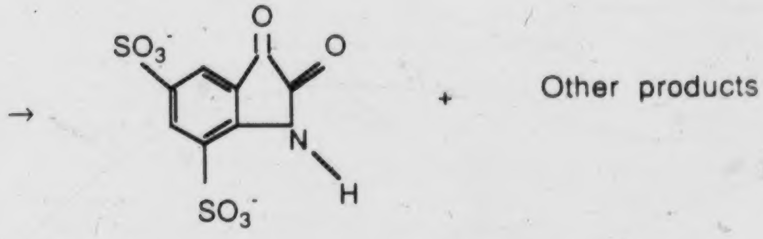
Researchers (Alder and Hill 1950; Gordon and Pacey, 1986; Ingols *et al.*, 1959; Kilpatric *et al.*, 1956) have shown that the maximum absorption of ozone, in aqueous solution using UV method, takes place at 260 nm which is in good agreement with the gas phase absorption of ozone at 253.7 nm. The significant differences, however, like ozone determination in the gas phase, are over the selection of an exact value of molar absorptivity which is determined by using an iodide method. The reported values of this coefficient lie in the range of 2,900 L/mole-cm at 258 nm (Bader and Hoigne, 1981; Kilpatrick *et al.*, 1956) to 3,600 L/mole-cm at 260 nm (Bahnemann and Hart, 1982; Fornl *et al.*, 1982). Further, the use of UV method to determine molecular ozone in water is subject to more interferences. The main interferants are dissolved organic and inorganic materials, aquatic humic substances, many oxidants like chlorine and bromine which absorb UV light at or near wavelength range as ozone does. Level of turbidity is another severe limitation in the use of this method. When turbidity level increases above 10 NTU, the light scattering effect influences the accuracy of this method (Johnson *et al.*, 1986; Stanley and Johnson, 1986). Thus, this method is applicable only to very pure solutions free from bacteria, turbidity and other absorbing materials, and, thereby, is mostly applicable in laboratories as a reference method where most of the time, ultrapure water is used, but it can not be used for natural waters and wastewaters.

In the Indigo method of determining residual ozone in water and wastewater, the decolorization of the sulfonated indigo dye is measured. This reagent reacts exclusively with ozone molecules with a very high reaction rate constant of 10^7 L/mole cm. Ozone reacts with only the -C=C- double bond in the indigo molecule (Hoigne and Bader, 1986). Decolorization depends upon the cleavage of this bond to produce a colourless product. Thus the change in absorbance of the indigo solution is proportional to the concentration of the ozone molecules. Because this is a bleaching method, the precision of the method depends upon the initial concentration of the indigo dye (Stanley and Johnson, 1986). This method is also subject to some interferences from oxidation products, like hydrogen peroxide, which reacts with ozone very slowly and also act as radical chain reaction carriers and thus promotes a catalytic decomposition of ozone (Bader and Hoigne, 1982). Similarly the presence of manganese and chlorine may interfere in the precise determination of ozone using this method. These interferences, however, can easily be removed by e.g. lowering the pH and by making use of glycine and malonic acid, respectively (Bader and Hoigne, 1982). Gordon *et al.* (1988) favour this procedure over all methods for the residual ozone determination due to its sensitivity, accuracy, precision, speed, and simplicity. Gordon and Pacey (1986) have concluded that indigo method appears to be an "ideal method" for determining aqueous ozone concentration.

Typically, potassium indigo trisulfonate dye is used which gives maximum absorbance at 600 nm with a molar absorption coefficient of 20,000 L/mole-cm and results in the production of isatin sulfonic acid (with molar absorption coefficient of 0 at 600 nm) (Bader and Hoigne, 1982) as:



Indigo trisulfonic acid



Isatin sulfonic acid

2.6 ROLE OF HYDROXYL RADICALS IN OZONE DISINFECTION

There is still a controversy over whether ozone in its molecular form is responsible for disinfection or whether hydroxyl radicals do this job. One of the reasons why ozone is so effective in disinfection may be its high oxidation potential of +2.07 volts. Hoigne and Bader (1976, 1978) proposed two reaction mechanisms of ozone in water and described that during ozonation part of the ozone dissolved in water. On one hand, ozone reacts directly with the soluble and suspended impurities. These direct reactions are highly selective and may take several minutes depending upon the nature of the impurities. On the other hand, part of the ozone added is decomposed and mainly hydroxyl radicals are produced. These radicals are highly reactive, react very fast with many types of dissolved species and so are entrapped by these impurities before they encounter a dispersed particle. Therefore, the oxidation of particulate matter becomes a low yield process in a non-purified system like wastewater. However, in many systems, the hydroxyl radicals react with solutes to form secondary intermediates of low reactivity (such as peroxy radicals) which, for their part, may survive until encountered with dispersed particles and become significant until lead to reaction. Therefore, when disinfecting with ozone under conditions which cause appreciable prior ozone decomposition, the hydroxyl radicals first form secondary intermediates which in turn react with the microorganisms even when solutes are present in low concentrations. Hence, the disinfection takes place by direct "fast reactions" of ozone and due to the reactions of secondary intermediates with microorganisms and not by "indirect reactions" of hydroxyl radicals themselves.

In contrast, other group of researchers differ from these findings and base their logic on the high oxidation potential (2.8 volts at unit hydrogen ion) of hydroxyl radicals (Baxendale, 1964; Rice and Taylor, 1986). Morris (1970), however, stated that

> "there is no relationship between the oxidation potential of a substance and its germicidal activity, but it can be said that substances that don't posses a high oxidation potential will not be germicidally active. In other words, chemical species that have high oxidation potential may posses high germicidal potential".

Bollyky (1977) claimed that the oxidizing power of a chemical usually parallels its disinfecting power. They both are reflected in its oxidizing potential and "disinfection occurs simultaneously with other oxidation reactions that consume ozone". Baxendale (1964) suggested that because of its high oxidation potential, hydroxyl radicals might be the species possessing strong germicidal activity in the ozonated water and not the ozone in its molecular form. Dahi (1976) related the ozonation efficiency directly with the ozone decomposition products e.g. hydroperoxyl, hydroxyl and ozone free radicals, and named them as "proper oxidative and germicidal agents".

From the literature, it can be established that a similarity exists between the action mechanism of ozone and hydroxyl radicals towards different organic compounds. For example, Hewes and Davison (1971) and Bishop *et al.* (1968) reduced the organic carbon content from wastewater samples using ozone and hydroxyl radicals respectively and got the same results. Similarly, when phenol was oxidized using ozone (Eisenhauer, 1968) and hydroxyl radicals (Stein and Weiss, 1951), they both gave the same intermediate products of catechol and o-quinone. Peleg (1976) concluded that the decomposition products of ozone appeared to be more powerful oxidizing agents than ozone. He further mentioned that

> "there is a similarity between the reactions of ozone and hydroxyl radicals with organic compounds and probably hydroxyl radicals are mainly responsible for the high oxidation potential of ozone in water. Thus, the hydroxyl radicals may be the potential source of high germicidal activity of ozone".

Hoigne and Bader (1979) stated that humic substances acted as hydroxyl radicals scavengers. Bancroft *et al.* (1984) postulated from these findings that since both humic substances and bacteria are similar in size, charge, functional groups, and gross morphology, therefore hydroxyl radicals should react with bacteria in the same way as they react with humic substances.

2.7 KINETICS OF DISINFECTION

Literature shows that the foundations of the kinetic approach to chemical disinfection were first developed, investigated and mentioned by Kroning and Paul in 1897, whose exemplary paper was later translated into English by Brock in 1961. After codifying rules which included the notion that comparative toxicity studies should be carried out at equimolecular proportions, with known number of bacteria in pure culture at constant temperature and under similar conditions of culture, Kroning and Paul applied the rules of chemical kinetics to the disinfection process, a process they stated was valid because the process must be a chemical one. They were the first to plot dose-response relationship in terms of the logarithm of the surviving organisms against time, which they found was linear in nature. Since then, the disinfection studies, in terms of dose-response relationship are still under continuous investigations by many researchers using different sets of conditions of disinfectants, organisms, medium, temperature, pH etc.

Chick (1908) used the same fundamental principles of reaction engineering, set by Kroning and Paul, in her experiments. Because the concentration of the disinfectant (phenol) was very high as compared to the density of bacteria in her experiments, so the it was regarded as pseudo constant during the process. From the data, she developed well known Chick's law. In its very simple form, this law is given as:

$$dN/dt = -kN$$

where

N = number of survivors,

dN/dt = rate of kill, and

k is a pseudo first order rate constant varying with the nature and concentration of the disinfectant.

Chick's law in which the organism is the only component changing concentration, expresses the rate of kill of microorganisms as an empirical first order model. Watson (1908) demonstrated that following relationship was also valid for Chick's data:

$$C^n t = \text{constant}$$

where,

C = disinfectant concentration,

t = time of disinfection, and

n = empirical constant, also known as coefficient of dilution.

Watson's law describes that when all other parameters are kept constant in a disinfection process, the disinfection efficiency depends upon the residual disinfectant concentration and residence time. By increasing one of these two variables and decreasing the other correspondingly, in such a way that their product remains constant, will give approximately same degree of disinfection. Using this concept, Collins *et al.* (1970) found that under well mixed conditions in a batch reactor, the reduction of coliform organisms in a chlorinated primary treated effluent could be described as:

$$N/N_0 = (1 + 0.23\ Ct)^{-3}$$

Where,

C = total chlorine residual at time t, mg/L, and

t = residence time, min.

Chick's law is valid under ideal conditions like:

1. The microorganisms and disinfectant are distributed uniformly,
2. Each microorganism is equally susceptible to a single species of disinfectant,
3. The disinfectant remains unchanged in chemical composition and constant in concentration during the reaction, and
4. Water is free from interfering substances.

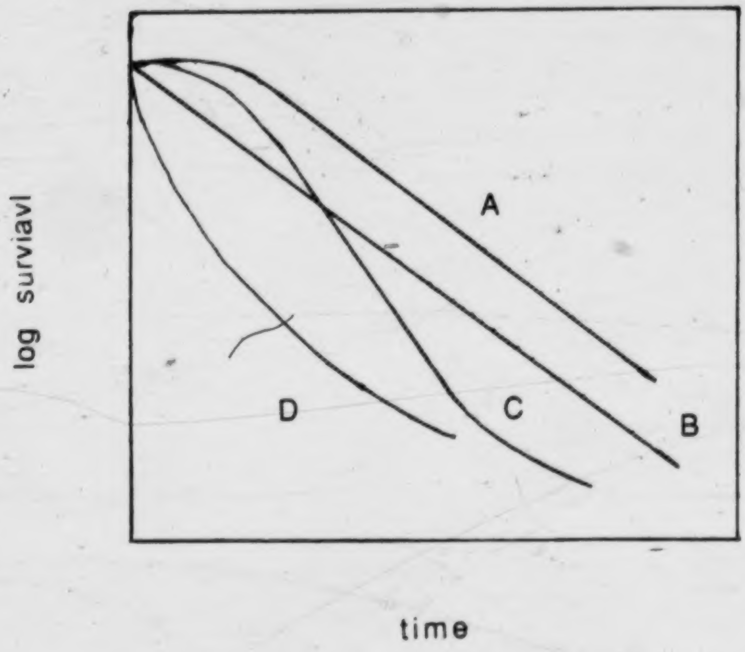
The departures from the Chick's law are not uncommon, even when test conditions are near ideal. In practice, these deviations may be due to many factors such as changes in disinfectant concentration with time, differences in resistance between individual organisms of various ages in the same culture, existence of clumps of organisms and occlusion of organisms by suspended solids (USEPA, 1986).

Normally, three types of deviations, from Chick's law, may occur; first in which time lag or shoulders are observed, second in which the rate of disinfection decreases with time and exhibit tailing effect, and third type of deviation is a combination of these two. Berg and co-workers (1988), after Moats (1971), showed these deviations as mentioned in Figure 2.3. A number of researchers have tried to explain these deviations. Fair *et al.* (1968) mentioned the increase in rate due to:

1. "The slow diffusion of chemical disinfectants through the cell wall and once enough disinfectant is accumulated within the cell, the rate of kill accelerates", and
2. "The consequence of a time lag before the disinfectant can reach a lethal number of vital centres in the organism."

Decrease in rate of kill is generally associated with the variation in cell resistance within the cell structure. However, declining concentration of disinfectant, poor distribution of organisms and disinfectant, and other interfering factors may account for it. Berg *et al.* (1988) postulated that an initial lag (curve A in Figure 2.3) in death rate can be attributed to multiple targets necessary for inactivation. They interpreted curves C and D due to the presence of heterogeneous population. Chang (1971), in agreement with Berg *et al.* (1988), explained that the presence of shoulders in a disinfection process are due to the availability of more than one target points in a bacterial cell, the disinfectant molecules have to react with and damage before the bacterial cell dies. Haas (1981), on the other hand, argued this as a time during which the disinfectant molecules diffuse through the outer layers of the cells which is necessary to inactivate the microorganisms. The tailing effect is explained by Poduska and Hershey (1972) and Cerf and Metro (1977) that "this is due to the presence of different types of populations with different sensitivities, inherent differences in sensitivities, or by the induction of resistance in survivors with time".

Fig. 2.3 Some types of survivor curves observed in disinfectin (adapted from Moats, 1971)



Attempts have been made to describe the time lag phase or shoulders, in chlorine disinfection, by semiempirical models. One such model was based on Chick (1908) and Watson (1908) laws, and was mentioned by Haas and Kara (1984) as "Chick-Watson's model", which is:

$$\log N/N_o = -kC^n t$$

where,

N/N_o = ratio of survived microorganisms at time t,

C = disinfectant concentration, which is constant, and

k and n are empirical constants.

Haas (1981) mentioned another model as:

$$\log N/N_o = -kC^n t^m$$

m and n are empirical constants. Typically, for free chlorine, n is near to 1, and the value of m is in the range of 2.3 to 3.2. Earlier, Hom (1972) developed a similar model to describe the deviations from Chick's law:

$$dN/dt = -kNC^n t^m$$

If m is less than 1, the rate of kill decreases with time; if m is greater than 1, the rate of kill increases with time. If n is greater than 1, time is more important than the dosage; if n is less than one, dosage is more important than time; and for n=1, effects of time and dosage are about the same. Under conditions such that m = 0, n = 0; this equation is reduced to Chick's law:

$$dN/dt = -kN$$

Haas (1980) formulated a model to predict the existence of a lag phase prior to onset of logarithmic decay of viruses using HOCl, for a system having negligible chlorine demand and under well mixed conditions. His approach was based on the formation of an intermediate reversible disinfectant-organism complex that controls the rate of microbial inactivation, and can be expressed as:

$$C + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} CS \xrightarrow{k_2} \text{dead cell}$$

C and S represent disinfectant molecule and receptor site, respectively, and CS is intermediate reversible disinfectant-organism complex. The assumptions in this reaction were:

1. the concentration of disinfectant remains constant,
2. the binding sites per organism, β, are uniform, and
3. both viable and killed organisms bind disinfectant in a uniform and constant manner.

Using this concept, Haas (1980) developed following model:

$$\ln N/N_o = -(k_2 C\beta/(C+K_D)) * \{ t + (\exp{-k_1 t(C+K_D)}-1)/(k_1(C+K_D))\}$$

In this model, $K_D = k_{-1}/k_1$ is known as "Michaelis constant". The time lag is given by $1/k_1(C+K_D)$, and the rate of inactivation which is equal to the slope of the linear portion of the plot of ln (N/N_o) vs. t, is equal to $k_2 C\beta/(C+K_D)$.

Haas and Kara (1984) used data obtained by other researchers to determine the best fit of Chick (1908), Chick-Watson (1908) and Haas (1980) models. The survival fractions (ln N/N_o) were analyzed as a function of C and t for each temperature and was concluded that Chick-

Watson model gave adequate fit of the inactivation data for free and combined chlorine and the other two models did not produce any statistically significant improvement in the correlation coefficient.

In ozone disinfection, like chlorination, the first step is the transfer of ozone into the aqueous medium. Once it is transferred, the residual oxidants such as ozone, hydroxyl radicals, or peroxide, must make contact with the organism to inactivate them. Therefore, similar requirements and kinetic relationships, as used for chlorine disinfection, can also be used for ozone disinfection (USEPA, 1986). However, still a major problem in the efficient application of ozone is the lack of complete and in depth knowledge of its kinetics. This results mainly from the poor understanding of the fundamental actions of ozone when in contact with different types of impurities in water. A number of researchers have developed different models to describe ozone disinfection kinetics taking into account different parameters; but like ozone decomposition studies, these models have been obtained under uncompareable conditions.

Katzenelson *et al.* (1974) demonstrated that ozone disinfection kinetics were different from those predicted for chlorine disinfection. They put greater emphasis on the effects exerted on viruses, because viruses were more resistant to disinfectants than bacteria; and investigated that ozone disinfection had two stages; an initial rapid stage followed by a much slower second stage. Stage one lasted less than 10 seconds during which a kill of about 99% was achieved; and second stage continued for several minutes during which final destruction occurred. Ozone was applied at seven intermediate levels between 0.07 and 2.5 mg/dm^3 but the phenomena was independent of the changes in the concentration.

Venosa *et al.* (1979) showed that the amount of ozone transferred was directly related to the number of coliforms entering the disinfection system. Stover and Jarins (1980) reported a relationship between transferred ozone and effluent coliform content and found that filtered nitrified effluent required less ozone transfer than conventional secondary effluent to achieve a given coliform standard. Given and Smith (1979) developed a mathematical model by multiple regression analysis and indicated that survival of indicator organisms could be predicted by knowing the amount of ozone transferred and BOD of the secondary effluent. Temperature of the effluent was another parameter they included in their model which was:

$$N/N_o = 9.2 \times 10^{-5}\ C_u^{-2.8}\ BOD^{1.9}\ T^{1.1}$$

where,

N/N_o = survival fraction,

C_u = ozone utilized in the range 2.5 to 35 mg/L,

BOD was in the range 45 to 220 mg/L, and

T = 5.5 to 14°C.

Venosa *et al.* (1981), similarly, developed empirical models which described that effluent total coliform numbers and fecal coliform numbers could be determined from the ozone transferred and the total chemical oxygen demand (TCOD) of the effluent. The relationship were:

$$TC = 24{,}000\ (TCOD)^{0.04} / T^{4.58}$$

$$FC = 6{,}610\ (TCOD)^{0.036} / T^{4.69}$$

where,

TC = total coliform/dL after ozonation,
FC = fecal coliform/dL after ozonation, and
T = ozone transferred, mg/L.

Caverson *et al.* (1986) developed two equations for their disinfection data of wastewater analogous to Venosa *et al.* (1981) and got:

$$TC_{effluent} = 10^{9.51}\ (BOD_5)^{0.0144} / T^{3.66}$$

$$FC_{effluent} = 10^{7.36}\ (BOD_5)^{0.0247} / T^{3.70}$$

Contact time is another thoroughly investigated parameter in ozone disinfection. Many investigators (Gale, 1943; Smith, 1969; Sproul *et al.*,1979) were of the opinion that ozone disinfection was relatively independent of contact time. Some studies (Stover *et al.*,1980), however, reported that effective disinfection could occur at contact time as short as one minute. Jekel (1982) mentioned that the contact time necessary for total disinfection varied from 5 to 10 minutes. USEPA (1986) summarized that isolating contact time as a particular parameter may not be of significant value because effective ozone disinfection is due to to the combined result of "high transfer efficiency, good mixing, adequate contact time and minimal short circuiting" in the reactor; and recommended from 6 to 10 minutes based on hydraulic considerations rather than process kinetics. In the field most existing systems have contact times from 10 to 15 minutes.

Finch (1987) obtained following regression model for his data on ozone dose-response in phosphate buffer, which included contact time but was applicable only in the ozone dose range of 45 to 810 μg/L:

$$\log N/N_0 = -0.427 - 1.72 \log C_A - 0.013\ t$$

where,

C_A = applied ozone dose (μg/L), and

t = disinfection time (sec).

This model gave a lack-of-fit below a dose level of 45 μg/L. Another example of the limitation of such a model can be given from Venosa *et al.* (1981) work. In this case, when Meckes *et al.* (1983) used a dose-response model, developed by their co-workers during their early work (Venosa *et al.*,1981), it gave lack of fit for high effluent COD's.

This discussion reveals that different disinfection models obtained by different researchers satisfy very limited range of variations in the parameters and no one model can be accepted as a standard. Therefore there is a need to look into the selection of variables and formulate a model which accommodates the variations in the parameters over a wide range.

2.8 SELECTION OF THE TEST ORGANISM

Escherichia coli (E. coli) has been chosen as the test bacterium because it is the most common organism used as an index of pollution in water and wastewater analyses and the assay techniques are standardized and relatively simple (Leland and Berg, 1988). Further, since it originates solely in mammalian guts, therefore, if present in a water and wastewater sample, provides an excellent and specific indication that true fecal contamination of the sample exists with its implied health hazards (Metcalf and Eddy, 1979; Perrich *et al.*, 1976).

2.9 SELECTION OF GROWTH MEDIUM FOR *E. coli*

Standard Methods (APHA, 1985) recommends the use of m-Endo agar or m-Endo LES agar as a growth medium for the recovery of coliform bacteria, with membrane filtration technique, at 35°C and 24 h incubation time. m-FC agar is recommended for use to recover fecal coliforms after 24 h incubation time at 44.5°C. Many researchers (Dutka, 1973; Evans *et al.*, 1981; Finch, 1987; Mcfeters *et al.*, 1982; Schiff *et al.*, 1970) have pointed out that these agars underestimated the numbers of injured coliforms and that there was very poor differentiation between coliforms and noncoliforms. Keeping in view the short comings of these media, LeChevallier *et al.*, 1983) proposed m-terigitol 7 (m-T7) agar to be used for the recovery of injured coliform bacteria from disinfected water. They proved that m-T7 agar recovered 86 to 99% more laboratory injured coliforms than did m-Endo agar. Using the same agar, they recovered nearly three times more coliforms than did m-Endo LES agar from drinking water. In an other study, LeChevallier *et al.* (1987) observed the growth of 18 different strains of *E. coli* using different kinds of media and showed that high recoveries were obtained with m-T7 agar at 35°C for 24 h incubation time. Growth conditions were kept same on all media. Mcfeters *et al.* (1986) compared the recovery of injured coliforms from drinking water using m-Endo LES agar and m-T7 agar at 35°C. Penicillin was added in m-T7 agar, even then m-T7 agar yielded 8 to 10 times more coliforms than m-Endo LES agar. In a recent study, Finch (1988) compared the ability of different media to recover stressed *E. coli* from ozonated waters and found that m-T7 agar at 35°C gave best recovery. All this suggests that the use of m-T7 agar yields the recovery of maximum number of stressed *E. coli.* Therefore, m-T7 has been selected to use as a growth medium in this study.

3. MATERIALS AND METHODS

3.1 ANALYTICAL METHODS

3.1.1 Phosphate buffer solution:

To prepare 0.05 M phosphate buffer solution at different pH values, appropriate amounts (appendix 1) of potassium dihydrogen phosphate (BDH analytical grade; mol. wt. 136.09) and disodium hydrogen phosphate (BDH analytical grade; mol. wt. 141.96) were dissolved in 1.5 L milli-Q water. The pH was checked with Fisher's Accumet Portable pH meter (Model 156) and was adjusted by diluted phosphoric acid (BDH analytical grade) or diluted sodium hydroxide (BDH analytical grade), as the case warranted.

The ozone was generated using extra dry oxygen in a chorona discharge ozone generator (Model C2P-9C-4), and its concentration in the oxygen carrier gas was monitored by a UV photometer monitor (Model HC12). Both instruments were from PCI Ozone Corp., West Caldwell, N.J. Operating the ozone generator at 110 volts, 41 Kpa (6 psi) back pressure, with an oxygen flow of 144 L/h (5 cu. ft./h) produced consistently 5.5 to 5.6% of ozone on weight to weight basis at standard conditions of temperature and pressure.

The ozone-oxygen gas mixture was bubbled through this solution for about 3 to 4 minutes followed by 5 minutes contact time to meet its ozone demand (Finch,1987). The flasks were autoclaved for 15 to 30 minutes, depending upon the autoclaving load, at 120°C to remove any residual ozone from the solution.

3.1.2 Indigo stock solution:

To prepare indigo stock solution, 1 mL of concentrated, analytical grade phosphoric acid (BDH) and 770 mg of potassium indigo trisulfonate (Aldrich Chem. Co.) were added to a 1 L volumetric flask containing 500 mL of milli-Q water with constant stirring. The flask was filled to the mark with milli-Q water. A 100 fold dilution of this solution exhibited an absorbance of 0.200 ± .010/cm at 600 nm. The stock solution was stored in a glass bottle in the dark at room temperature. Before making indigo reagents I and II, the absorbance of the stock solution was checked and it was discarded if the absorption of 100 fold dilution fell below 0.16/cm.

3.1.3 Indigo reagent I:

To a 1 L volumetric flask, added 20 mL of indigo stock solution, 11.50 g of analytical grade sodium dihydrogen phosphate, $NaH_2PO_4 \cdot H_2O$, and 7 mL of concentrated analytical grade phosphoric acid. The contents were diluted to the mark. The solution was discarded when its absorbance fell below 80% of its initial value.

3.1.4 Indigo reagent II:

The indigo reagent was prepared in the same way the indigo reagent 1 was made except that 100 mL of indigo stock solution was used instead of 20 mL.

3.1.5 Sodium thiosulphate solution:

To prepare stock sodium thiosulphate solution containing 1.0 mg $Na_2S_2O_3$/mL to use as an oxidant neutralizing agent, 392 mg $Na_2S_2O_3 \cdot 5H_2O$ was dissolved in 250 mL of milli-Q water. Five millilitres solution was dispersed into each test tube, and autoclaved for 20 min, for easy use and to avoid contaminating the whole lot. The test tubes were stored at room temperature and the contents of one test tube were used per litre of solution in reaction vessel.

3.1.6 Preparation of ozone stock solution:

To prepare ozone stock solution to be used during disinfection studies, 450 mL of phosphate buffer solution was placed in a 500 mL gas absorption bottle. The ozone-oxygen gas mixture was passed through phosphate buffer solution for at least one hour at room conditions. The absorption bottle was disconnected and the ozone concentration of the solution was determined.

3.1.7 Concentration of ozone stock solution:

Ten millilitres of indigo reagent II was placed in two 100 mL volumetric flasks, A and B, containing magnetic stirs. Flask A (blank) was filled to the mark with milli-Q water. Two millilitres of the stock ozone solution was injected into the flask B using Oxford's Macro Set pipette in such a way that completely decolourized zones were quickly eliminated by stirring but no ozone was degrazed through the air bubbles formation. After adding the ozone solution, the flask was filled to the mark with milli-Q water like flask A. A dual purpose visible-ultraviolet spectrophotometer (Model Spectronic 601, Milton Roy Company) was used to measure the absorbances of both the solutions at 600 nm in 10 mm cuvette. The ozone concentration of the solution was calculated using the following equation.

Conc. of O_3 (gm/L) =

$$\Delta A.\ v.\ (\text{mol. wt. of ozone}) \ /\ \varepsilon.\ d.\ \text{vol. of } O_3 \text{ sol. added}$$

where

$\Delta A = A_o - A$,

A_o = absorbance of the indigo reagent in flask A (blank),

A = absorbance of the indigo reagent in flask B,

v = total volume of the contents of the flasks, 100 mL,

ε = molar absorptivity at 600 nm (20,000 L/mole-cm),

d = path length = cuvette size (1 cm),

volume of ozone solution added to the flask B = 2 mL.

Thus,

ozone conc. (g/L) of the stock ozone solution =

$(\Delta A.\ 100\ mL.\ 48\ g/mole)/\ ((20{,}000\ L/mole\text{-}sec)(1\ cm)(2\ mL))$

or

$$\text{Concentration (mg/L)} = 240\ (\Delta A)$$

The above operating conditions of the ozone generator consistently gave ozone concentration in the range of 22 to 23.5 mg/L in phosphate buffer solution.

3.1.8 Determination of applied ozone dose:

Based on the concentration of the ozone stock solution, the volume of the ozone stock solution to be injected into the reaction vessel was calculated for a desired dose. Since the concentration of the ozone stock solution was consistently in the range of 22 to 23.5 mg/L and the applied dose was about 46 µg/L, therefore, 2 mL of the ozone stock solution was used to dose ozone in each reaction vessel for each experimental trial. However, to calculate the exact amount of the ozone dose to the reaction vessel, the concentration of the ozone stock solution was determined immediately before and after injecting the ozone dose into the reaction vessel using indigo method. The average of these two was taken as the actual concentration of the ozone stock solution at the time of dose and was used for subsequent calculations.

3.1.9 Residual ozone concentration:

To dertermine the residual ozone concentration in the reaction vessel after specified reaction time, 10 mL of indigo reagent II was placed in two volumetric flasks A and B containing magnetic stirs. Flask A (blank) was filled up to the mark with ozone demand free milli-Q water. After specified reaction time, an appropriate volume of the solution was drawn using a pipette and was injected into flask B. The flask was then filled to the mark using ozone demand free milli-Q water. The absorbances of the indigo reagents in both the flasks were determined and the difference between them was used, as mentioned in section 3.1.7, to calculate the residual concentration of ozone.

3.2 BACTERIOLOGICAL METHODS

3.2.1 Tryptone Soya Broth (TSB):

To prepare tryptone soya broth, appropriate amount of TSB (Difco Laboratories), in 30 g per 1 L milli-Q water basis, was rehydrated in an Erlenmeyer flask. The contents were heated slightly for complete dissolution, dispensed in the final container and autoclaved for 15 minutes at 120°C.

3.2.2 0.1% Peptone water:

To make 0.1% peptone dilution water, which improves the recovery of the injured coliform at room temperature (McFeters *et* *al.*,1982), Bacto peptone (Difco Laboratories) was dissolved as 1 g/L of milli-Q water in a 20 L bottle. The dilution water was dispensed in Nalgene dilution bottles using Brewer automatic pipetting machine (Scientific Equipment Products, Maryland) to give final volumes of 30, 90 and 99 mL after autoclaving for 30 min at 120°C.

3.2.3 Growth medium:

To prepare m-T7 agar plates, 33 g of medium (Difco Laboratories) was suspended in 1 L milli-Q water and heated to boiling to dissolve completely. It was autoclaved for 15 min at 120°C and cooled down to about 40 to 45°C before pouring into MF plates.

3.2.4 Incubation of *E. coli*:

The culturing and subculturing of *E. coli* was performed by incubating the seeded TSB at 37°C for 18 to 24 h. Similarly the growth of *E. coli* cells on the membrane filters was obtained at the same conditions.

3.2.5 Test organism:

Escherichia coli strain 11775 from the American Type Culture Collection (ATCC), which has been used by Finch (1987), was used in this study.

3.2.6 *E. coli* Densities

3.2.6.1 No ~ $10^{4.2}$ CFU/dL:

A loop full of *E. coli* from stored nutrient agar slant at 4°C was cultured into a test tube containing 5 mL tryptone soya broth (TSB) and was incubated at 37°C. After 18 to 20 h, a loop full of cultured *E. coli* was transferred into another tube containing 5 mL TSB and incubated at 37°C for another 18 to 20 h. For consecutive working, however, *E. coli* was inoculated from the sub-cultured test tube for 18 to 20 h at 37°C.

To obtain an initial density of $10^{4.2}$ CFU/dL; 2 mL of subcultured *E. coli* was transferred into a 5 mL centrifuge tube. A pellet of *E. coli* was obtained by centrifuging the suspension in a bench scale Sorvall centrifuge (Model SPX) for 30 min at 7500 rev/min (rpm). The centrifuge was allowed to stop by itself after this time. The liquid was poured off from the centrifuge tube; 2 mL of sterilized, ozone demand free 0.05 M phosphate buffer solution were added to the tube using Kimbell 5 mL sterilized disposable pipette. The pellet was resuspended using a sterilized disposable Pasteur transfer pipette and then vortexed for 5 sec for uniform and even resuspension. This procedure was repeated for three times to make sure that the bacterial culture becomes free from even trace amount of growth medium which otherwise reacts with the ozone applied and jeopardizes the results.

One millilitre of resulting suspension of *E. coli* was transferred into a sterilized glass bottle containing 99 mL of sterilized, ozone demand free 0.05 M phosphate buffer using 1 mL sterilized disposable pipette. The contents were shaken vigorously to ensure uniform distribution of the *E. coli* in the bottle and were further diluted 1:100 in another bottle containing 99 mL of sterilized, ozone demand free 0.05 M phosphate buffer solution. One millilitre of this mixture was pipetted into the reaction vessel containing 1 L sterilized, ozone demand free 0.05 M phosphate buffer solution and was stirred at moderate speed for about 2 min for thorough mixing before drawing samples to determine initial density of *E. coli* in the reaction vessel. Dilutions were made by adding 10 mL of this mixture into a dilution bottle containing 90 mL of sterilized 0.1% peptone water. The contents were shaken well and 1, 2.5 and 5 mL volumes of this ten-fold diluted suspension were pipetted into dilution bottles, in triplicate, containing 30 mL sterilized 0.1% peptone water. The contents were mixed by shaking the bottles and filtered according to standard membrane filtration procedure (APHA, 1985). Filters were placed on the m-terigitol 7 (m-T7) agar plates and incubated for 24 h at 37°C. After incubation time, colonies were counted. The acceptable range of colonies was 20 to 80 colony forming units (CFU) per plate for every dilution and their spread was checked by Poisson distribution (Eisenhart and Wilson, 1943; Haas and Heller, 1986). If the index of dispersion, D^2, was in excess of tabulated value of Ψ^2 statistics for the 5% level of significance, the counts were discarded. Using the counts which met these two conditions, the *E. coli* density in the reaction vessel was calculated using geometric mean of the replicates and was expressed as CFU/dL.

This technique gave a mean initial *E. coli* density of $10^{4.22}$ CFU/dL (n=12, standard deviation=0.025 log units) in the covered vessel and $10^{4.22}$ CFU/dL (n=12, standard deviation=0.018 log units) in the uncovered vessel.

3.2.6.2 No ~ $10^{7.2}$ CFU/dL:

To obtain an initial density of $10^{7.2}$ CFU/dL, 2 mL of subcultured *E. coli* suspension was washed and resuspended with 2 mL of sterilized, ozone demand free 0.05 M phosphate buffer solution in the same way as described in section 3.2.6.1. After final washing and resuspending the *E. coli* pellet, 1 mL of the suspension was pipetted into 99 mL sterilized, ozone demand free phosphate buffer solution. The contents were mixed thoroughly by shaking the bottle and 10 mL of this diluted suspension was transferred into the reaction vessel containing 1 L of sterilized, ozone demand free 0.05 M phosphate buffer solution. The contents were stirred for 2 min before drawing sample to determine initial density of bacteria in the reactor.

Two 1 mL samples were drawn from the vessel and pipetted into two dilution bottles containing 99 mL sterilized 0.1% peptone water. Contents were mixed by shaking bottles and another 100 fold dilution was made by introducing 1 mL of this mixture into 99 mL of sterilized 0.1% peptone water. 2 and 4 mL samples of 1:10,000 diluted suspension were drawn, in triplicate, and transferred into bottles containing 30 mL of sterilized ozone demand free peptone water for filtration, incubation and determining the initial density of the *E. coli* in the reaction vessel according to the procedure described in 3.2.6.1. The mean initial density of bacteria obtained using this procedure was $10^{7.2}$ CFU/dL (n=15, std. dev. 0.007 log units) in covered vessel and $10^{7.2}$ CFU/dL (n=14, std. dev. 0.012 log units) in uncovered vessel.

3.2.6.3 No ~ $10^{9.3}$ CFU/dL:

The cultured *E. coli*, after 18 to 24 h incubation in a 5 mL TSB at 37°C, was subcultured into two 5 mL test tubes. After specified incubation, the contents of the two TSB tubes were transferred into two centrifuge tubes and centrifuged and washed as mentioned in section 3.2.6.1 using 5 mL of sterilized, ozone demand free 0.05 M phosphate buffer for each tube each time. After final washing and resuspension, the contents of the two centrifuge tubes were poured into the reaction vessel containing 1 L of sterilized, ozone demand free 0.05 M phosphate buffer solution. The contents were allowed to stir for 2 min for uniform distribution in the vessel before taking sample to determine initial density of the *E. coli.*

Two one mL samples were drawn from the vessel and 1:1,000,000 dilutions were made in three steps using 1 mL into 99 mL of sterilized 0.1% peptone water. 1, 2 and 5 mL of finally diluted suspension were filtered using 30 mL of sterilized 0.1% peptone water as mentioned in section 3.2.6.1. The mean bacterial densities obtained were $10^{9.30}$ CFU/dL (n=15, std. dev. 0.021 log units) in the covered vessel and $10^{9.30}$ CFU/dL (n=15, std. dev. 0.018) in the uncovered vessel.

3.2.6.4 No ~ 10^{11} CFU/dL:

Initial calculations showed that *E. coli* subcultured in about 1 L TSB would be required to get an initial density of 10^{11} CFU/dL in the reaction vessel. For this purpose, *E. coli* was cultured into two 5 mL TSB tubes from nutrient agar slant and was incubated at the 37°C for 18 to 24 h. The cultured *E. coli* was, then, subcultured by pipetting 1 mL of cultured *E. coli* into five 250 mL Erlenmeyer flasks each containing 200 mL of TSB. Flasks were shaken well for uniform distribution of cultured organisms in the medium and thus for even and uniform growth. After incubation period of 18 to 24 h, the contents of the flasks were transferred into two 500 mL sterilized, ozone demand free Nalgene polycarbonate bottles and centrifuged for 15 minutes at 7500 rpm in a Sorvall RC-5B Refrigerated Superspeed centrifuge. After this time, the centrifuge was allowed to stop by itself. The liquid was poured off from the bottles and the bacterial pellet was resuspended in each bottle using 100 mL sterilized, ozone demand free phosphate buffer. A sterilized, ozone demand free graduated cylinder was used to transfer 100 mL of the buffer solution into the centrifuge bottles. This procedure was repeated three times to ensure that even the trace amount of organic growth medium was removed. After last washing and resuspension, the contents of the two centrifuge bottles were poured into the reaction vessel containing 800 mL of sterilized, ozone demand free phosphate buffer making the total volume in the reactor one litre. The contents of the reactor were allowed to stir for 5 min before taking the sample to determine the initial bacterial density in the reaction vessel.

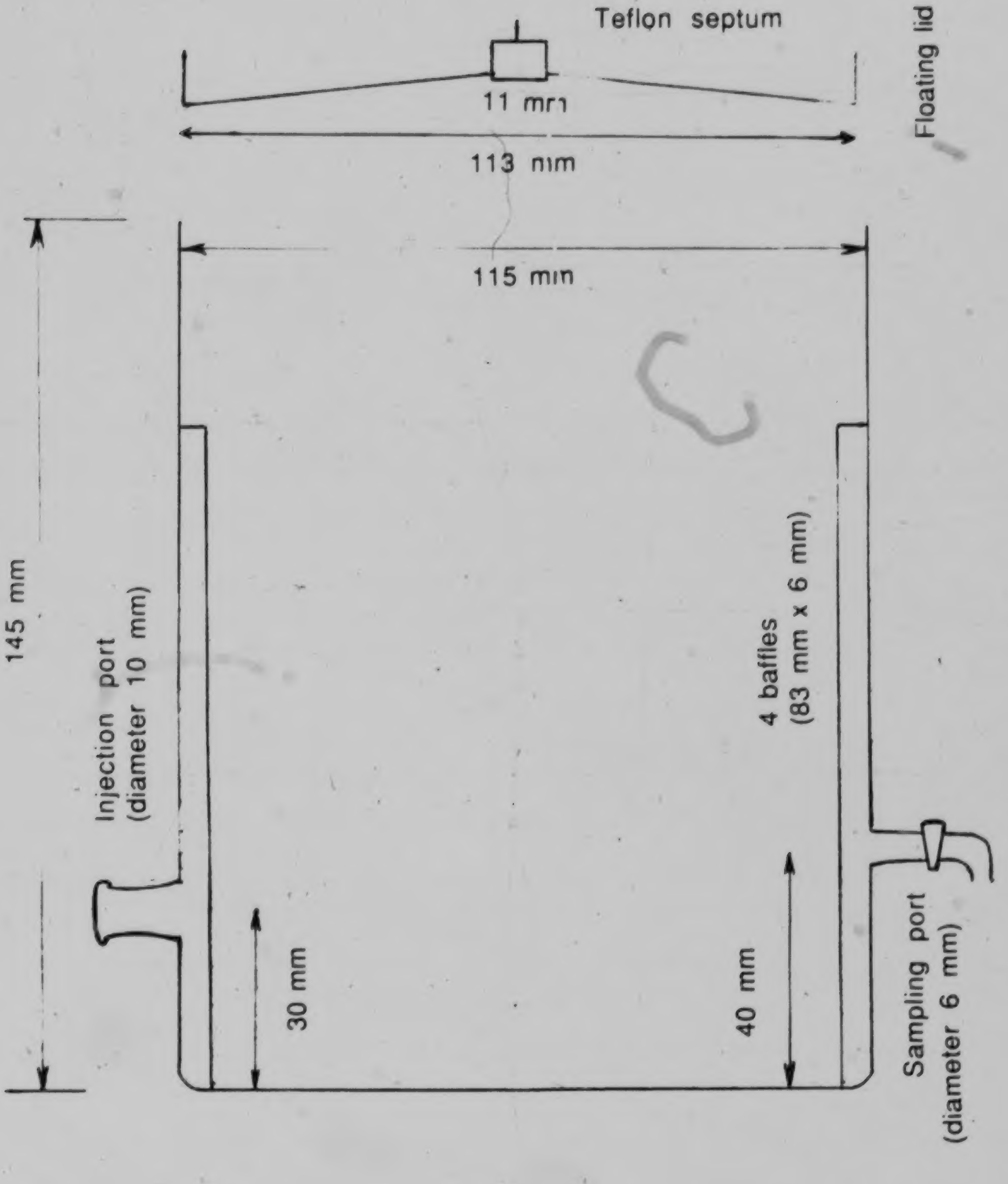
One mL sample was drawn from the reactor and diluted to 1:100,000,000 fold in four steps by pipetting 1 mL into 99 mL of sterilized 0.1% peptone dilution water. 0.1, 1, 2, 5 and 10 mLs of the finally diluted suspension were used, in triplicate, to determine the bacterial density according to the procedure mentioned in section 3.2.6.1. Only 5 mL plates gave counts in the acceptable range. The mean *E. coli* densities obtained were $10^{11.03}$ CFU/dL (n=12, std. dev. 0.025 log units) in the covered vessel and $10^{11.03}$ CFU/dL (n=12, std. dev. 0.061 log units) in the uncovered vessel.

3.3 REACTION VESSEL:

The reactor designed and fabricated for this study was made of glass with internal diameter of 115 mm and was 145 mm high. Four baffles were fixed in it to facilitate complete and uniform mixing of the reactor contents with a magnetic stir bar. A Teflon top of 113 mm external diameter, with the bottom sloped toward its centre to remove the entrapped air, was made in such a way that it floated on the surface of the liquid in the reactor but neither let the

Fig 3.1

REACTION VESSEL


Teflon septum
Floating lid
11 mm
113 mm
115 mm
145 mm
Injection port (diameter 10 mm)
4 baffles (83 mm x 6 mm)
30 mm
40 mm
Sampling port (diameter 6 mm)

ozone escaped from the system nor exerted any friction with the glass walls. This floating lid was used only when the reaction conditions were termed "covered". An 11 mm hole in the centre of the floating lid was used to inject the ozone doses into the system and to draw the samples for analysis. A Teflon septum was used to close this hole during reaction time. An additional injection port of 10 mm dia and a sampling port of 6 mm dia were provided about 30 mm and 40 mm from the bottom of the reaction vessel respectively. Teflon septums were used in these ports to block them.

3.4 PROCEDURES:

3.4.1 Ozone decomposition studies:

Two 450 mL volumes of sterilized, ozone demand free 0.05 M phosphate buffer solution, of desired pH value, were transferred into two 500 mL gas absorption bottles using sterilized, ozone demand free 500 mL graduated glass cylinder. Ozone-oxygen gas mixture was passed through these bottles for about 1 h. One hundred millilitre of the same phosphate buffer solution was poured into a sterilized, ozone demand free reaction vessel containing a magnetic stir bar. The contents were stirred continuously but taking care not to create turbulent vortex in the solution. The ozonated buffer solution was poured into the reaction vessel carefully so that no ozone gas was escaped to the atmosphere in the form of bubbles. The sterilized, ozone demand free Teflon floating lid was placed on the solution if the decomposition studies were under "covered" conditions. The ozone disappearance in this case was only through auto-decomposition. However, when lid was not placed on the solution, the disappearance of ozone was through auto-decomposition as well as due to stripping to the atmosphere. The initial concentration of this solution in the reactor was determined by drawing a sample using macro set pipette (Oxford) and injecting into 10 mL of indigo reagent II in 100 mL volumetric flask. To determine the disappearance of ozone over time, samples were taken at 0, 2, 5, 10, 20, 30, 45, 60 and 120 min. The disappearance of ozone was studied in 0.05 M phosphate buffer solution at three pH values (4, 6.9, 9) using both covered and uncovered systems.

3.4.2 Dose-response studies:

3.4.2.1 Bacterial die-off in phosphate buffer solution:

To observe the effects of phosphate buffer solution on *E. coli* survival, 1 L of sterilized, ozone demand free 0.05 M phosphate buffer solution, at desired pH, was poured into the reaction vessel using sterilized, ozone demand free graduated cylinder. The floating lid was placed on the solution if the reaction conditions were "covered". Appropriate amount of washed *E. coli* suspension was injected into the the solution using a sterilized, disposable pipette. The contents were mixed continuously using a sterilized, ozone demand free magnetic stir bar. After 2 min of mixing, samples were drawn using a sterilized disposable pipette. Appropriate dilutions were made using sterilized 0.1% peptone dilution water. After filtration, using membrane filtration technique, filter papers were placed on m-T7 agar plates and incubated for 18 to 24 h at 37°C. Appropriate dilution factor was used to transform the counts data into CFU/dL. The samples were drawn at 0, 15, 30, 45 and 60 min to observe any adverse effects of buffer solution on the *E. coli* survival over time. Phosphate buffer solution was used at three pH levels (4, 6.9 and 9) for three initial *E. coli* densities ($10^{4.20}$ CFU/dL, $10^{7.20}$ CFU/dL, $10^{9.30}$ CFU/dL) using both covered and open reaction vessels. The bacterial die-off studies with $N_o \sim 10^{11.0}$ CFU/dL could not be conducted due to time limitations.

3.4.2.2 Bacterial die-off in phosphate buffer solution in the presence of sodium thiosulphate solution:

Because 0.1% sodium thiosulphate solution was used in the dose-response experiments to neutralize excess ozone at the end of the specified reaction time, it was appropriate to study the effects, of the addition of this solution into phosphate buffer solution, on the bacterial population. One litre of sterilized, ozone demand free 0.05 M phosphate buffer solution was poured into the reaction vessel as mentioned in section 3.4.2.1. Five millilitres of sterilized, ozone demand free 0.1% sodium thiosulphate solution was added to the buffer solution. The lid was placed for "covered" conditions. Appropriate amount of washed *E. coli* culture was injected into the reaction vessel with a sterilized, disposable pipette. The contents were continuously stirred with a magnetic stir bar for 2 min before first sample was drawn to determine initial density of bacteria.

Other samples were drawn at 15 and 30 minutes from covered and uncovered reaction vessels. This study was conducted only in 0.05 M phosphate buffer solution at pH 6.9 with an initial bacterial density ~ $10^{7.2}$ CFU/dL.

3.4.2.3 Ozone dose - *E. coli* kill studies:

To determine the effects of applied ozone dose on the bacterial population, 1 L sterilized, ozone demand free 0.05 M phosphate buffer solution (800 mL for $N_o \sim 10^{11.0}$ CFU/dL) was poured into the reaction vessel as described in section 3.4.2.1. Teflon lid was placed on the solution to provide "covered" conditions. Appropriate volume of washed bacterial suspension was pipetted into the phosphate buffer solution using a sterilized, disposable pipette. The contents were mixed continuously for uniform distribution of *E. coli* in the solution and to provide equal chances of contact between bacterial suspension and ozone molecules after applying ozone dose. After 2 min (5 min for $N_o \sim 10^{11.0}$ CFU/dL), a sample was drawn using 1 mL sterilized, disposable pipette to determine initial density of *E. coli* in the reaction vessel.

Four hundred and fifty millilitres of the same type of phosphate buffer solution was placed in a 500 mL gas absorption bottle and stock ozone solution was made by passing ozone-oxygen gas mixture for about 1 h. Two millilitres of this ozonated buffer solution were injected into the reaction vessel with macro set pipette (Oxford). Immediately before and after injecting ozone solution into the reaction vessel, its concentration was determined using indigo reagent II. The applied ozone dose was based on the average of these two concentrations. After specified reaction time, a 25 to 50 mL sample was drawn, using a tip cut, calibrated, 50 mL pipette, from the vessel to determine the residual ozone concentration using indigo reagent I. Sodium thiosulphate was added to neutralize residual ozone. The difference of ozone applied and residual ozone was the amount of ozone utilized during the reaction. Sample were drawn to determine the final density of the *E. coli* in the reaction vessel. The difference of the initial and final densities was the kill due to the amount of ozone utilized. The experiments were conducted at random using four initial *E. coli* densities ($10^{4.2}$ CFU/dL, $10^{7.2}$ CFU/dL, $10^{9.3}$ CFU/dL and $10^{11.0}$ CFU/dL) at three pH levels (4, 6.9 and 9) using "covered" and "uncovered" conditions in the reaction vessel. The applied ozone dose was kept constant at 46 µg/L.

3.5 QUALITY CONTROL

3.5.1 Ozone demand free water and buffer solution:

The milli-Q water and phosphate buffer solutions were made ozone demand free and sterilized according to the method described in section 3.1.1.

3.5.2 Ozone demand free glassware:

Any glassware which could be used in ozone dose - bacterial response experiments was washed with phosphoric acid and rinsed twice with deionized water at 85°C in Miele automatic washer (Model G7733). It was then soaked in a 20 L neck-cut bottle, containing strong ozone solution, for about 1 h. Glassware was dried and autoclaved to remove excess ozone traces and was cooled to room temperature before using.

3.5.3 pH adjustments of buffer solutions:

pH adjustments of the buffer solutions were made according to the method mentioned in section 3.1.1.

3.5.4 Calibration of pipettes:

Pipettes were used quite extensively to transfer bacterial cultures and to draw samples for analyses. To check that the delivered volumes were accurate, 8 pipettes, 2 from each one of the four different brands (Corning, P4249-10, graduated 10 mL; Corning, P4184-10, volumetric 10 mL; Pyrex, 7065, graduated 10 mL; Kimble, disposable, graduated 5 mL), were picked at random. The volumes delivered by these pipettes were weighed using Sartorius-Werke balance (Model 6MBH) and compared with the masses of the same volumes calculated by using density at 21°C. Two types of water (milli-Q water and sterilized, ozone demand free milli-Q water) were used to calibrate the pipettes. It was observed that

- generally volumetric pipettes delivered less volumes than graduated pipettes,
- graduated pipettes with 10 mL marks well above the tips (Pyrex and Corning) delivered more volumes than those with exact volume marks (Kimble brand),
- statistically there was no significant difference between the mass delivered by these pipettes and the mass of the liquids calculated using a water density at 21°C (0.998 gm/L) for 1% significance level.

3.5.5 Control experiments:

Die-off studies were conducted to observe any adverse effects of phosphate buffer solution and sodium thiosulphate solution on the bacterial suspension over time and no such effects were found over short period. Initial density of *E. coli* was determined for each experimental run to give an accurate survival ratio in dose-response experiments after the reaction time.

3.5.6 *E. coli* culturing and subculturing:

Generally, the *E. coli* was cultured and subcultured around 5 p.m. and was used around 11 a.m. next morning, thus giving bacterial cultures of the same relative ages throughout the experimentation.

3.5.7 Nutrient agar plates streaking:

To confirm that bacterial cultures were free from any contamination, nutrient agar plates were streaked from time to time. The *E. coli* colonies grew on these plates did not show any sign of contamination in the stored *E. coli* slant.

3.5.8 Centrifugation and washing of *E. coli*:

The subcultured *E. coli* in TSB was centrifuged and washed three times to ensure that even the trace amounts of organic matter were removed which otherwise could react with the ozone dose applied and jeopardize the results.

3.5.9 Determination of N_0 and N:

Throughout the study, two different volumes were used to determine the number of bacterial counts. Only one volume gave counts in acceptable range, the counts from other volume, however, gave an estimation of the precision of the methodology.

3.5.10 Randomization:

It was always possible that mistakes might be made due to unsuspected sources of disturbances which might result in inaccurate and biased conclusions. Randomization of the experiments was the solution of this problem (Box *et al.*,1978) because it allowed every experimental unit an equal chance of receiving equal treatment and, thus, deduced causality and unspecified disturbances and gave unbiased estimates. Hence, randomization was implemented in the selection of the experimental runs, in making dilutions and in doing filtrations.

3.5.11 **Statistical analysis**:

Only that microbiological data were considered acceptable, which fulfilled the following criteria,

- the plate counts were in the range of 20 to 80, and
- the acceptable counts followed Poisson probability distribution.

For this purpose, D^2 statistics (Eisenhart and Wilson, 1943; Haas and Heller, 1986) was applied, which is described as:

$$D^2 = (n - 1)\, S^2 / X_{\text{geo. mean}}$$

Where,

D^2 = index of dispersion,
n = no. of replicates,
S = standard deviation,
S^2 = an estimate of population variance, and
$X_{\text{geo. mean}}$ is an estimate of population mean.

The calculated index of dispersion, D^2, was compared with the tabulated value of chi-square, ψ^2, statistics for (n-1) degree of freedom at 5% level of significance. If the comparison showed that dispersion was in excess of Poisson (i. e. $D^2 > \psi^2$ (a, n-1)), then the variation in the counts was not by chance only and the data was not accounted for calculations.

4. RESULTS AND DISSCUSION

4.1 EFFECTS OF pH ON OZONE DECOMPOSITION:

A total of 17 independent trials were conducted using 0.05 M phosphate buffer solution in covered and uncovered reaction vessels at pH 4, 6.9 and 9. The mean initial concentration of applied ozone in the reactor was 21 mg/L (std. dev. 0.43). The raw data is tabulated in appendix 2. The covered reaction vessel gave the rate of ozone decomposition while the combined decomposition and stripping rate to the atmosphere was achieved in the data obtained from uncovered vessels.

To analyze the reaction rate data, three different techniques, algebraic, differential and integral, were used. However, the latter two were normally used to develop reaction rate equations for the data obtained from batch operations which is in the form of concentration vs. time (Grady and Lim, 1980). Roth and Sullivan (1983) pointed out that because the ozone decomposition followed a pseudo first order kinetics, a simple power law equation could be used to analyze the data from ozone decomposition studies. Roth *et al.* (1979) mentioned that this equation was applicable under constant volume, batch and isothermal conditions. The general form of the rate equation is:

$$r = dC/dt = -kC^n \qquad \text{(equ. 4.1)}$$

The integral forms of this equation are:

for $n = 1$

$$\ln C = \ln C_o - kt$$

or $$\log C = \log C_o - (kt/2.303) \qquad \text{(equ. 4.2)}$$

for $n \neq 1$

$$C^{1-n} = C_o^{1-n} + (n-1)\,kt \qquad \text{(equ. 4.3)}$$

These rate equations are analogous to the general form of the straight line equation $y = mx + a$, therefore, should give straight lines when right hand side of equations are plotted against 't' for the assumed valve of n. The $(-k/2.303)$ and $(n-1)$ are slopes and $\log C_o$ and C_o^{1-n} represent y-intercepts for equations 4.2 and 4.3, respectively

The integral method was used to determine the order of the equations by assuming the n values as 0, 0.5, 1.0, 1.5 and 2 and the data obtained during the ozone decomposition experiments was plotted as mentioned above in the Figures 4.1 to 4.3. The plots with $n = 1$ found to describe the data satisfactorily where the correlation coefficient for the least square fit were always higher than those determined for $n = 0, 0.5, 1.5$ and 2 and ranged from 0.993 to 0.999. It was also observed that the pH of the solutions and reaction vessel conditions did not influence the order of the ozone decomposition in the range of pH 4 to 9.

To observe the effects of stripping of ozone to the atmosphere, t-test was applied to C/C_o values with respect to time from covered

Fig. 4.1 (A-1)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 4, n=0)

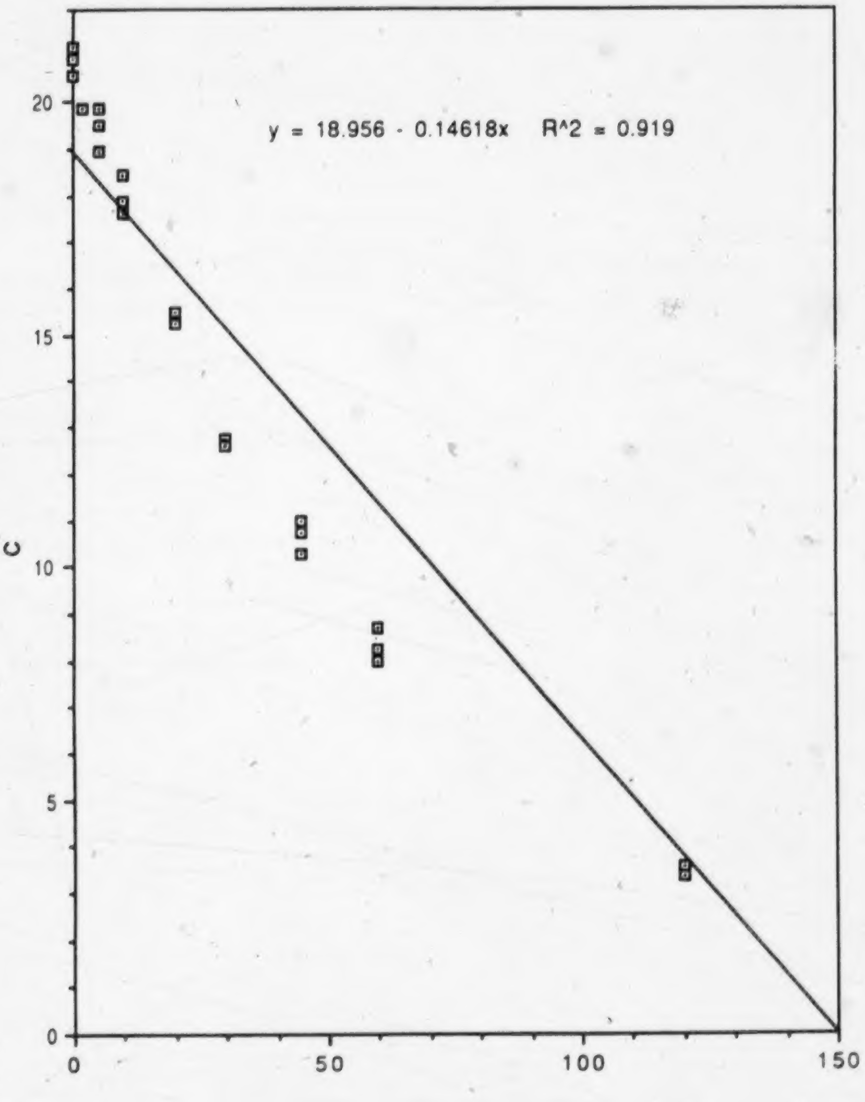


Fig. 4.1 (A-2)

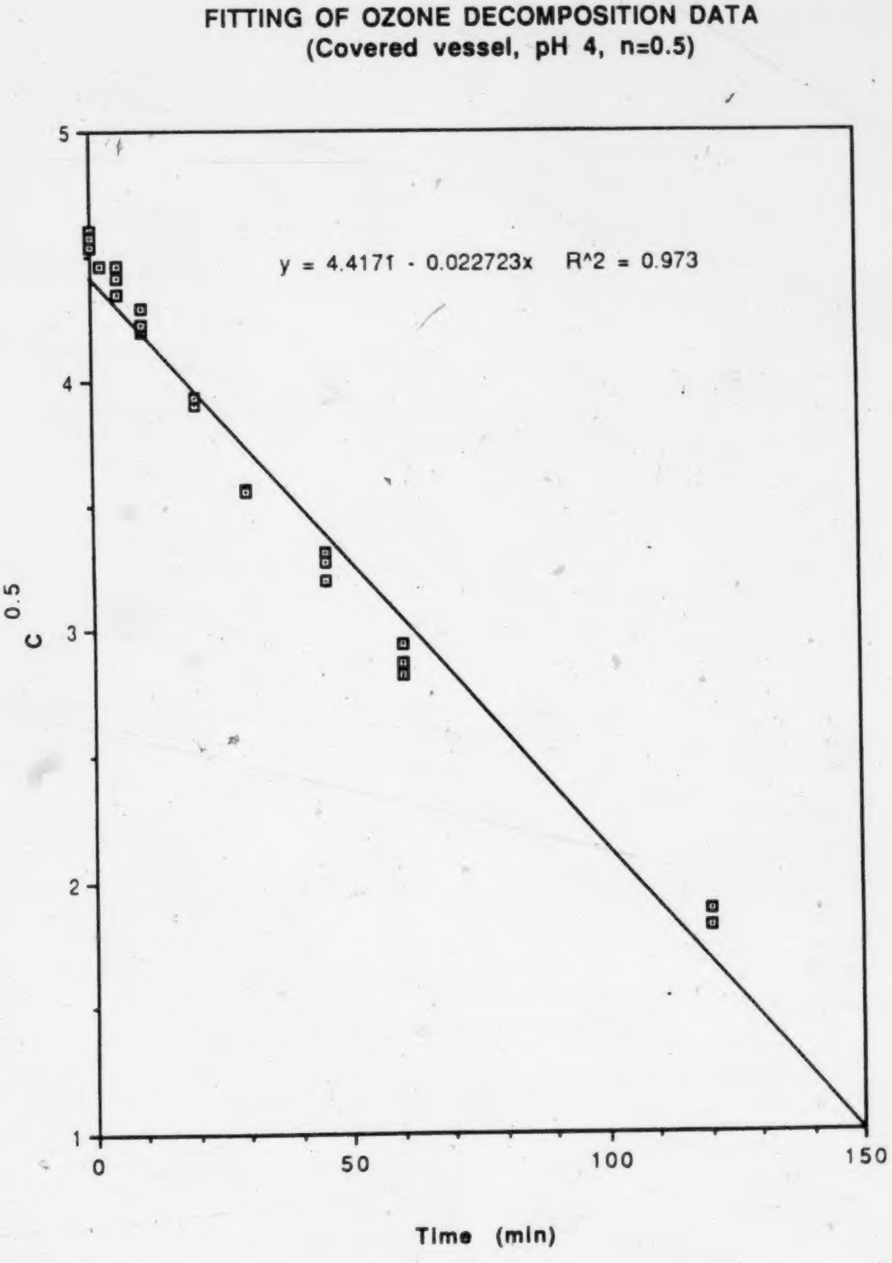

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 4, n=0.5)
y = 4.4171 - 0.022723x R^2 = 0.973
C 0.5
5
4
3
2
1
0
50
100
150
Time (min)

Fig. 4.1 (A-3)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 4, n=1.0)

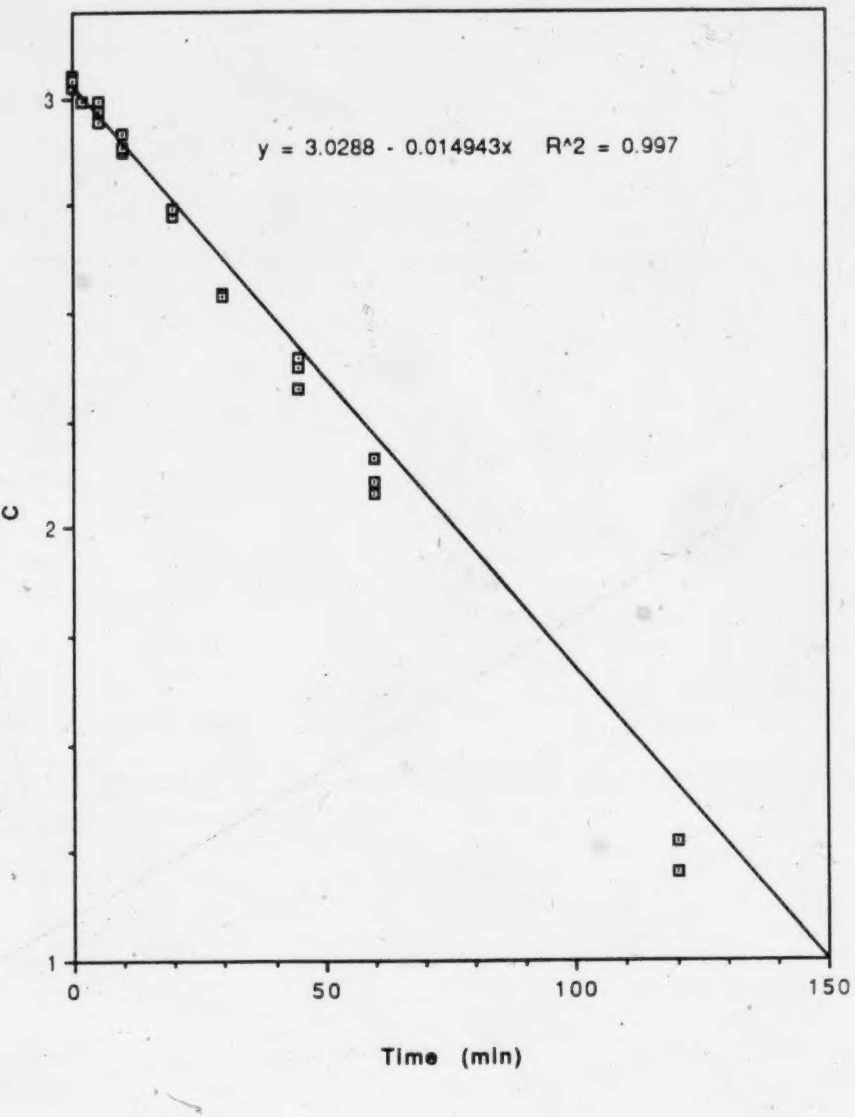


Fig. 4.1 (A-4)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 4, n=1.5)

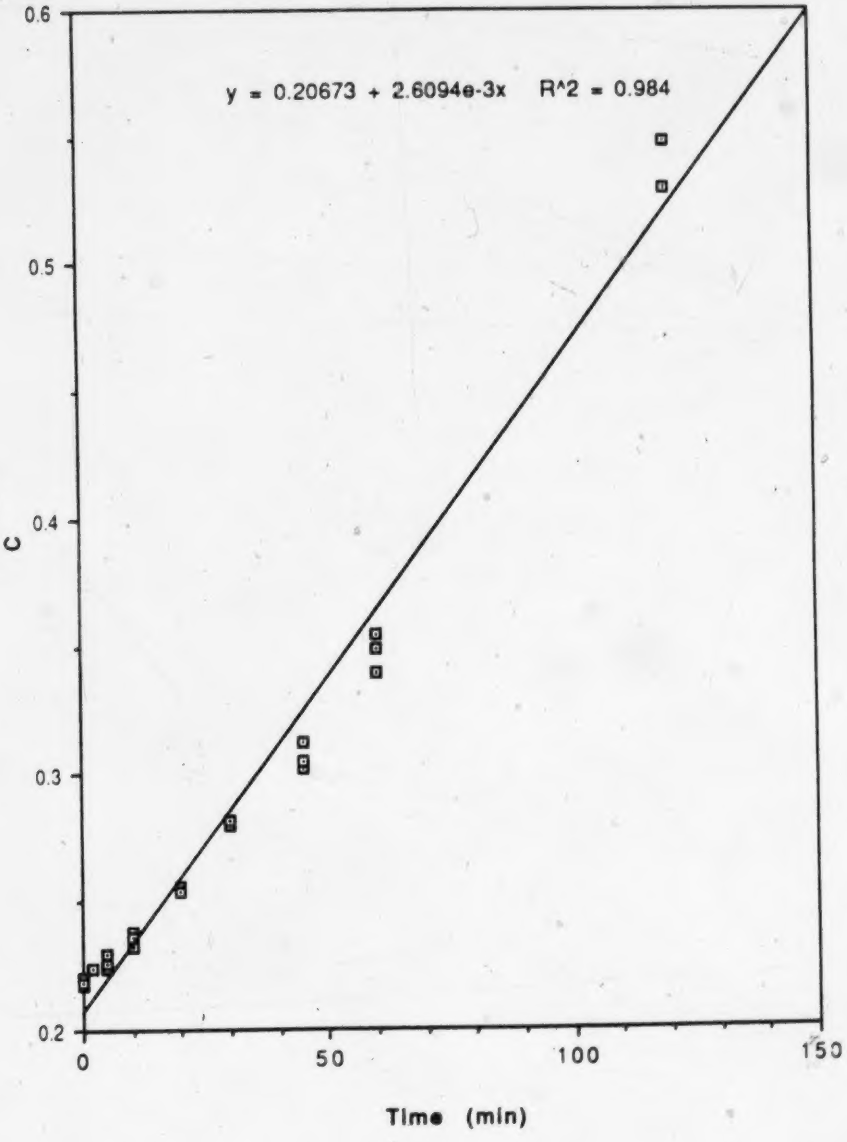


Fig. 4.1 (A-5)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 4, n=2.0)

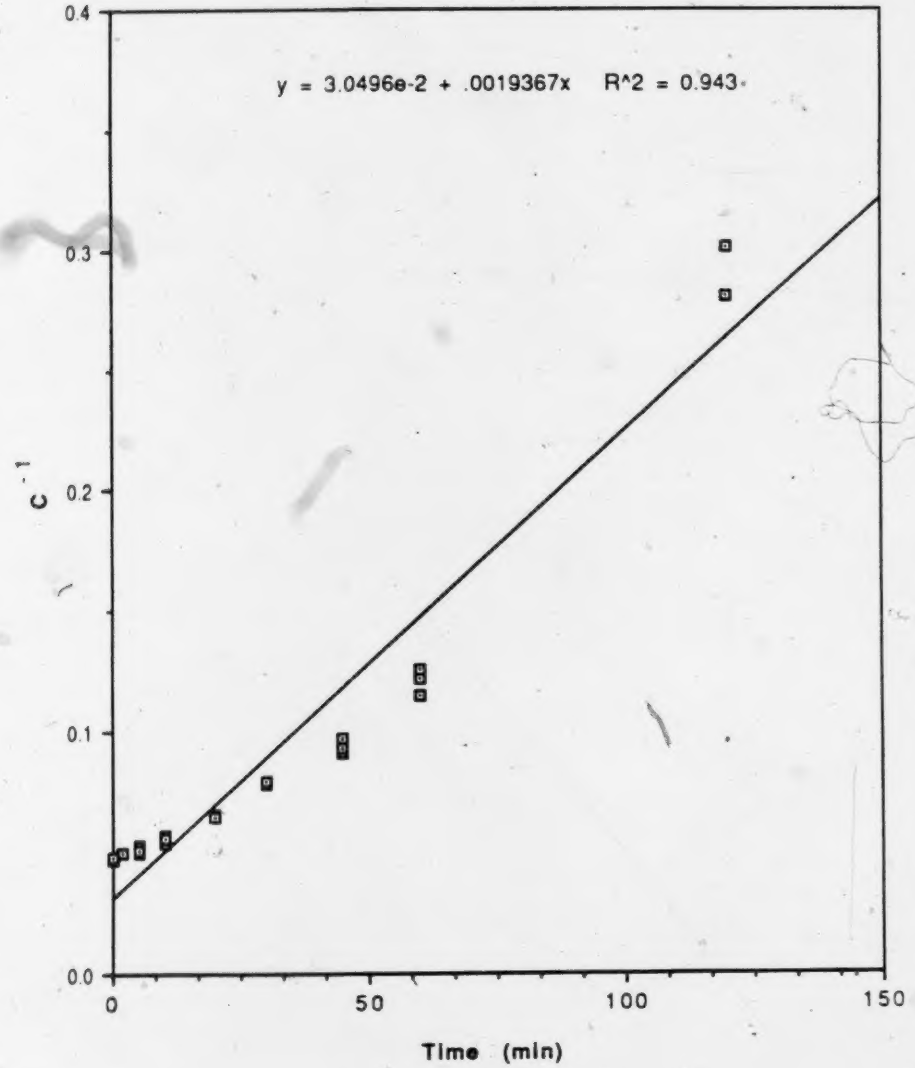


Fig. 4.1 (B-1)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 4, n=0)

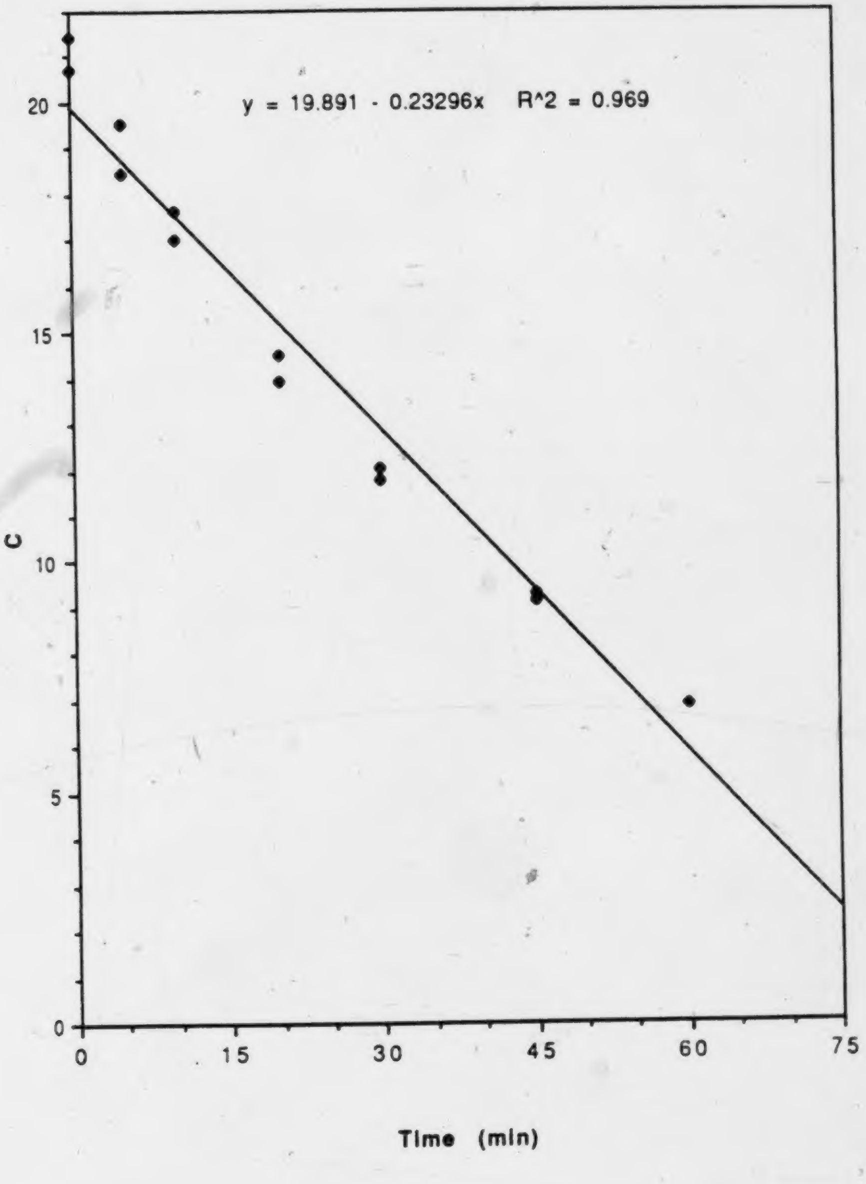


Fig. 4.1 (B-2)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 4, n=0.5)

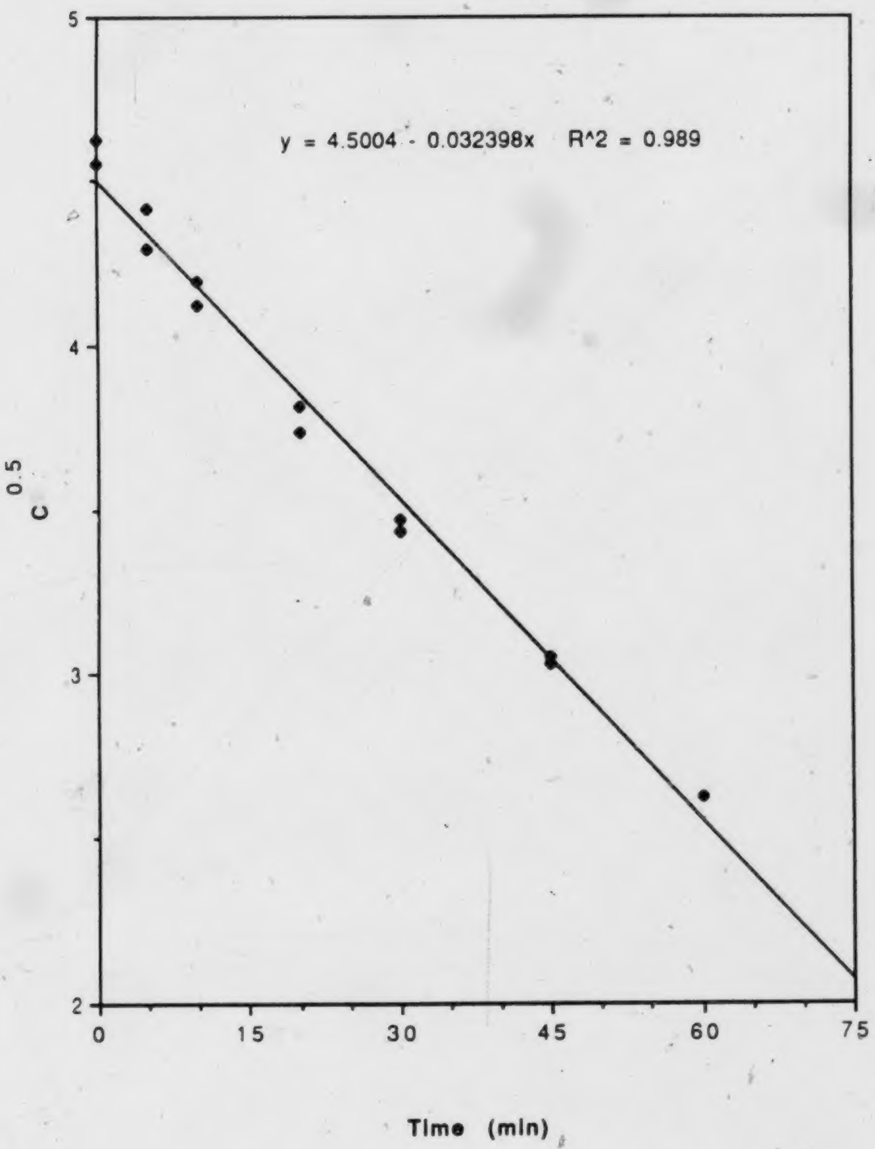


Fig. 4.1 (B-3)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 4, n=1.0)

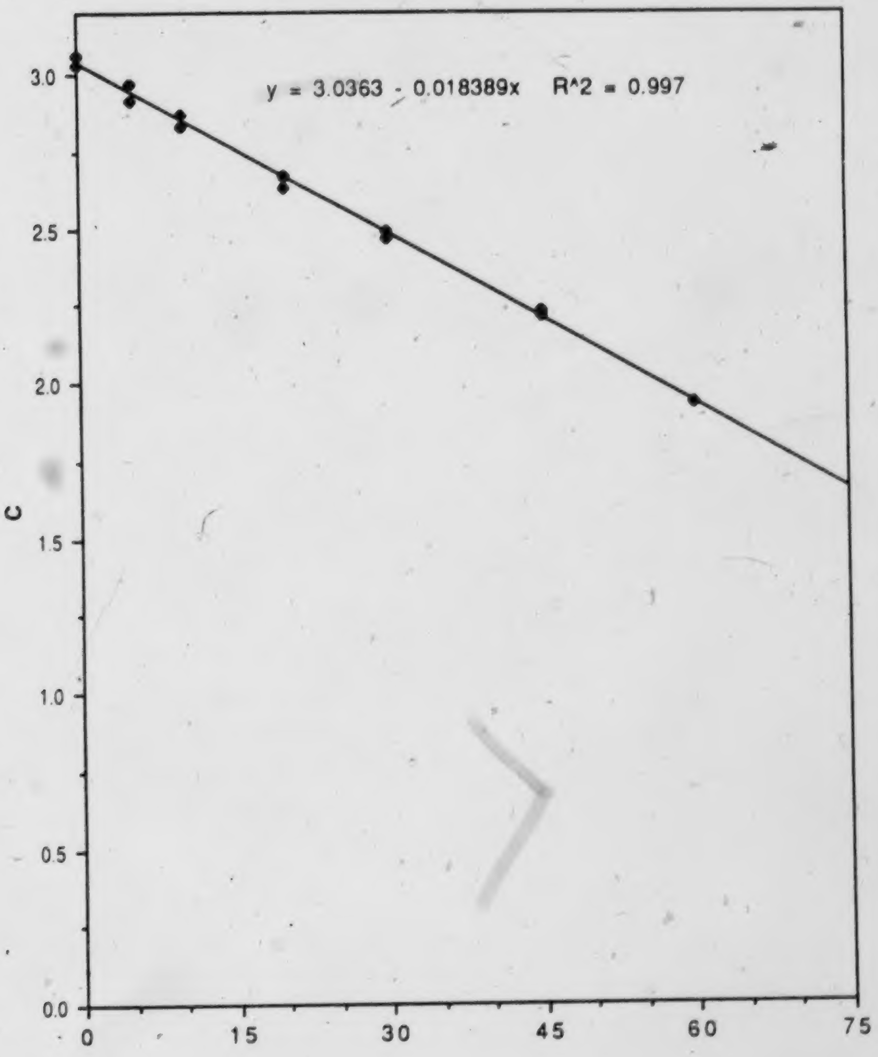


Fig. 4.1 (B-4)

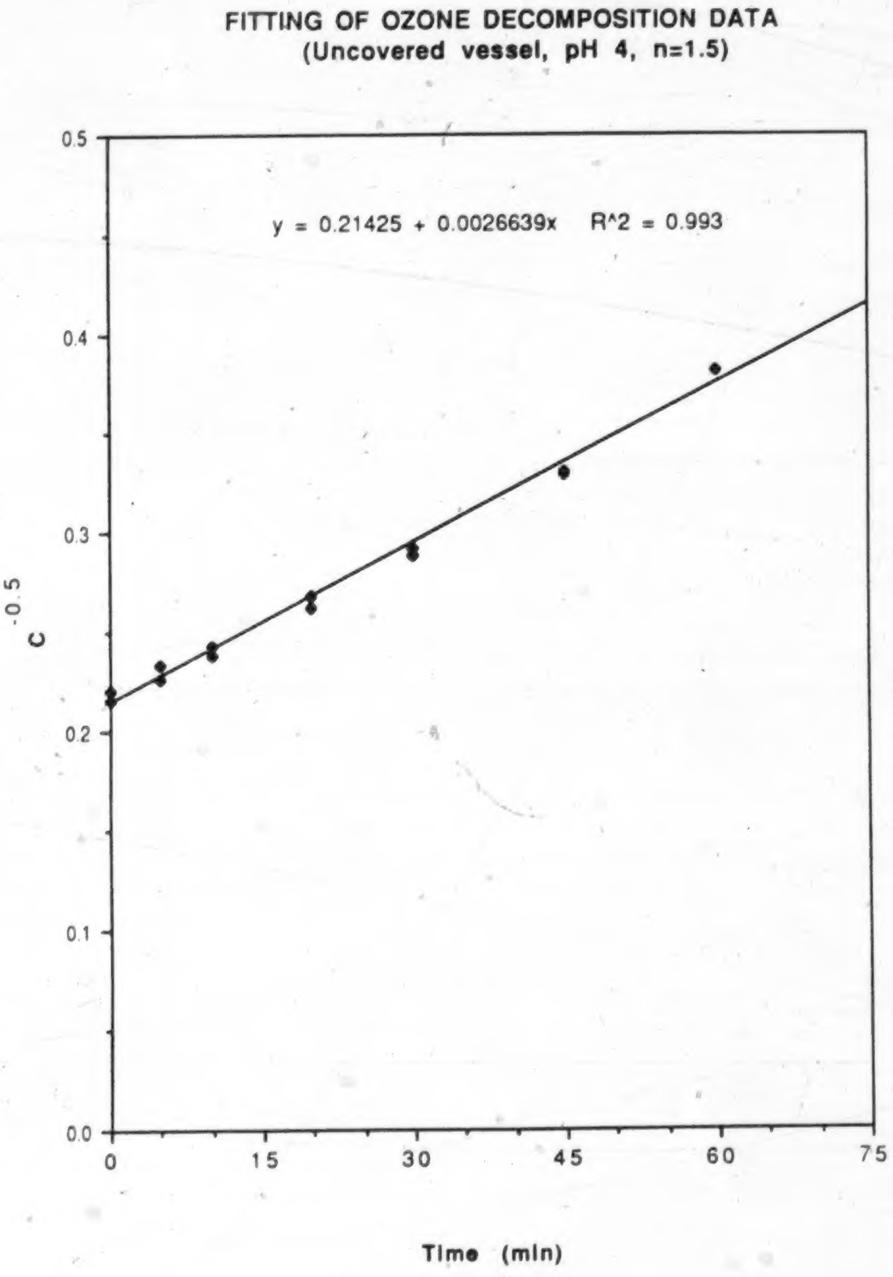

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 4, n=1.5)
y = 0.21425 + 0.0026639x R^2 = 0.993
0.5
0.4
0.3
0.2
0.1
0.0
$C^{-0.5}$
0
15
30
45
60
75
Time (min)

Fig. 4.1 (B-5)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 4, n=2.0)

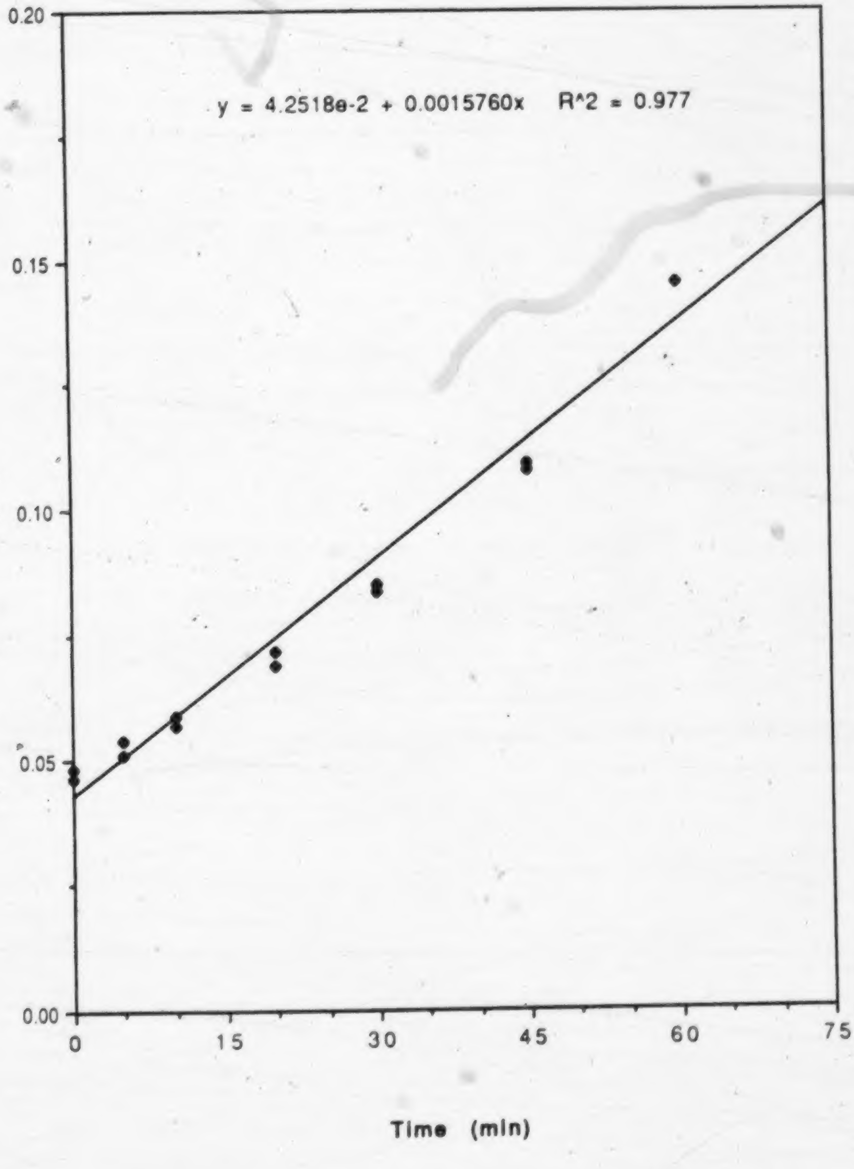


Fig. 4.2 (A-1)

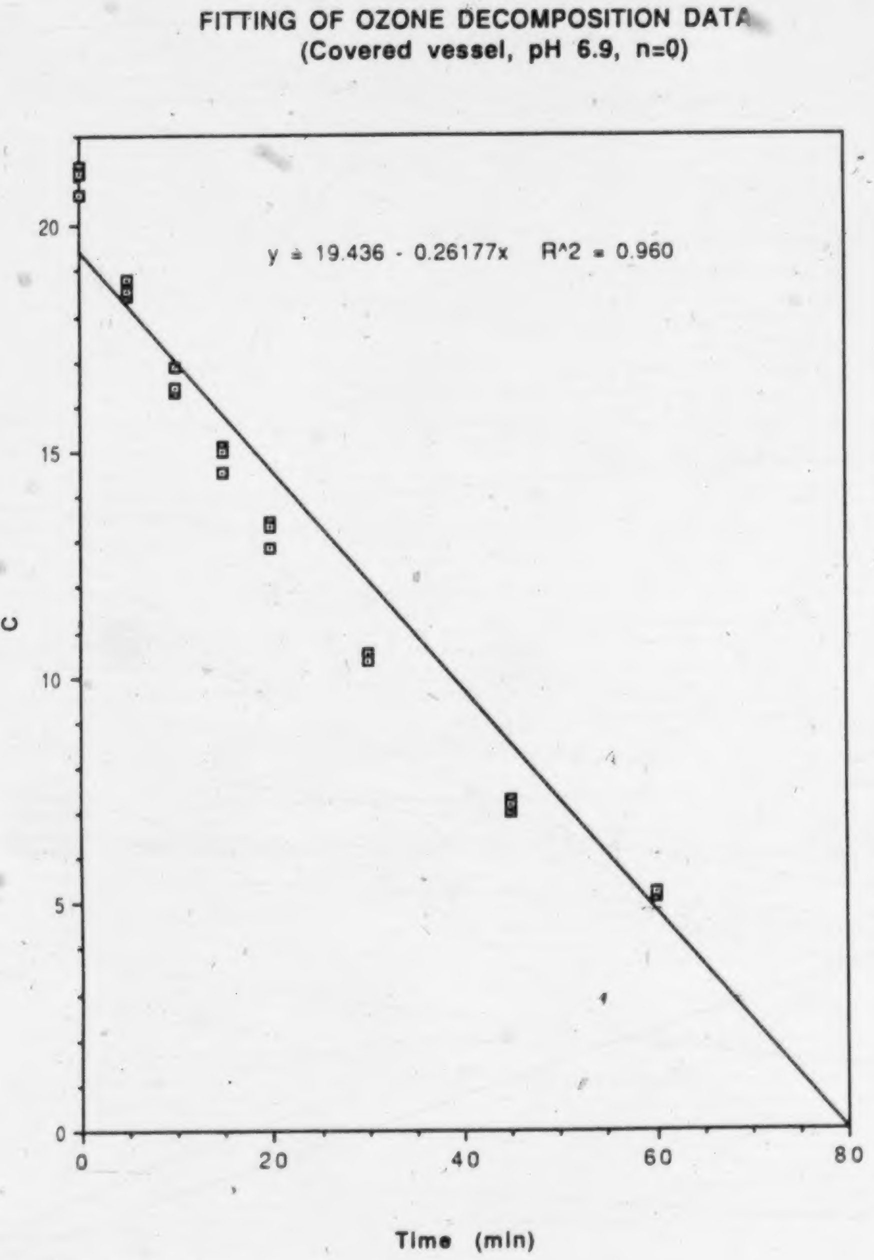

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 6.9, n=0)
y = 19.436 - 0.26177x R^2 = 0.960
C
20
15
10
5
0
0
20
40
60
80
Time (min)

Fig. 4.2 (A-2)

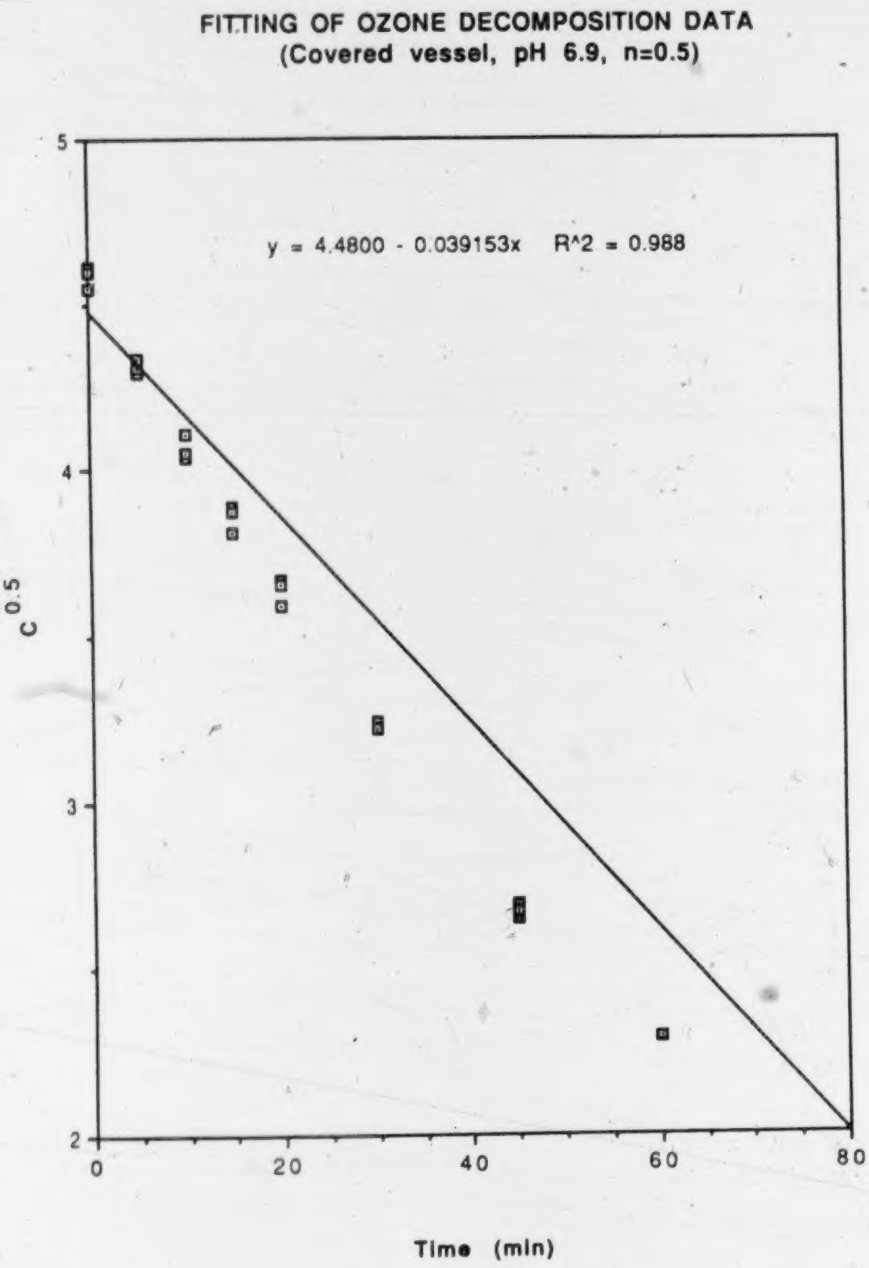
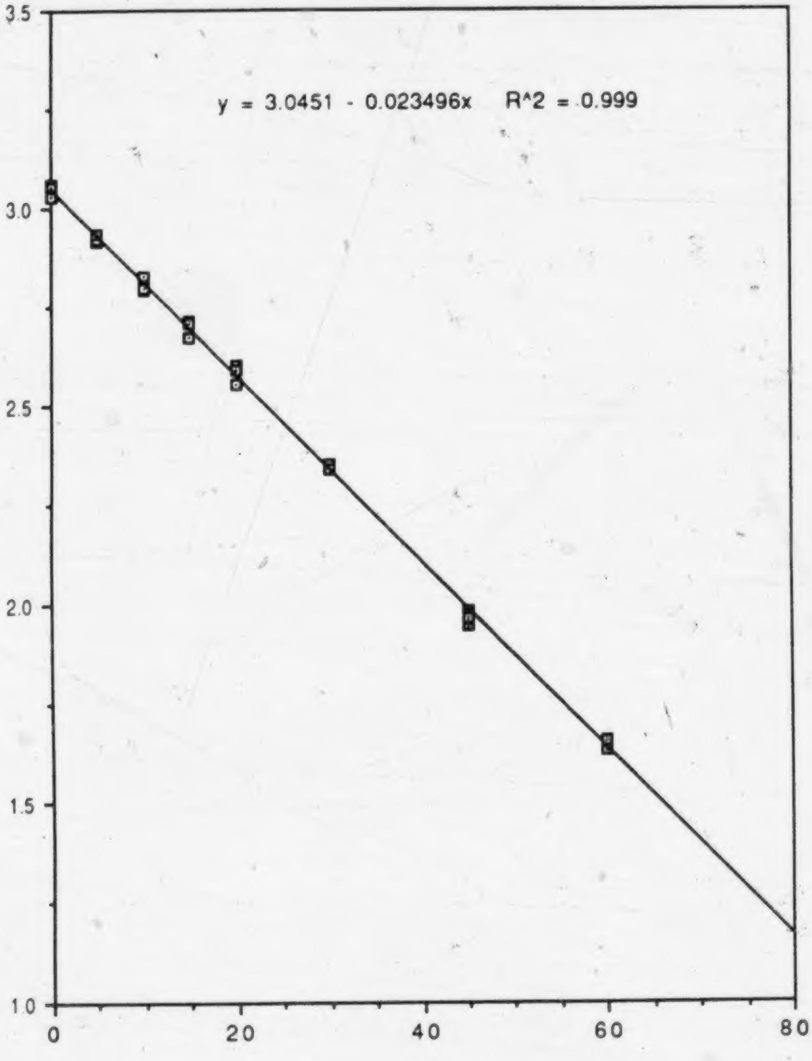

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 6.9, n=0.5)
y = 4.4800 - 0.039153x R^2 = 0.988
$C^{0.5}$
5
4
3
2
0
20
40
60
80
Time (min)

Fig. 4.2 (A-3)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 6.9, n=1.0)



Time (min)

Fig. 4.2 (A-4)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 6.9, n=1.5)

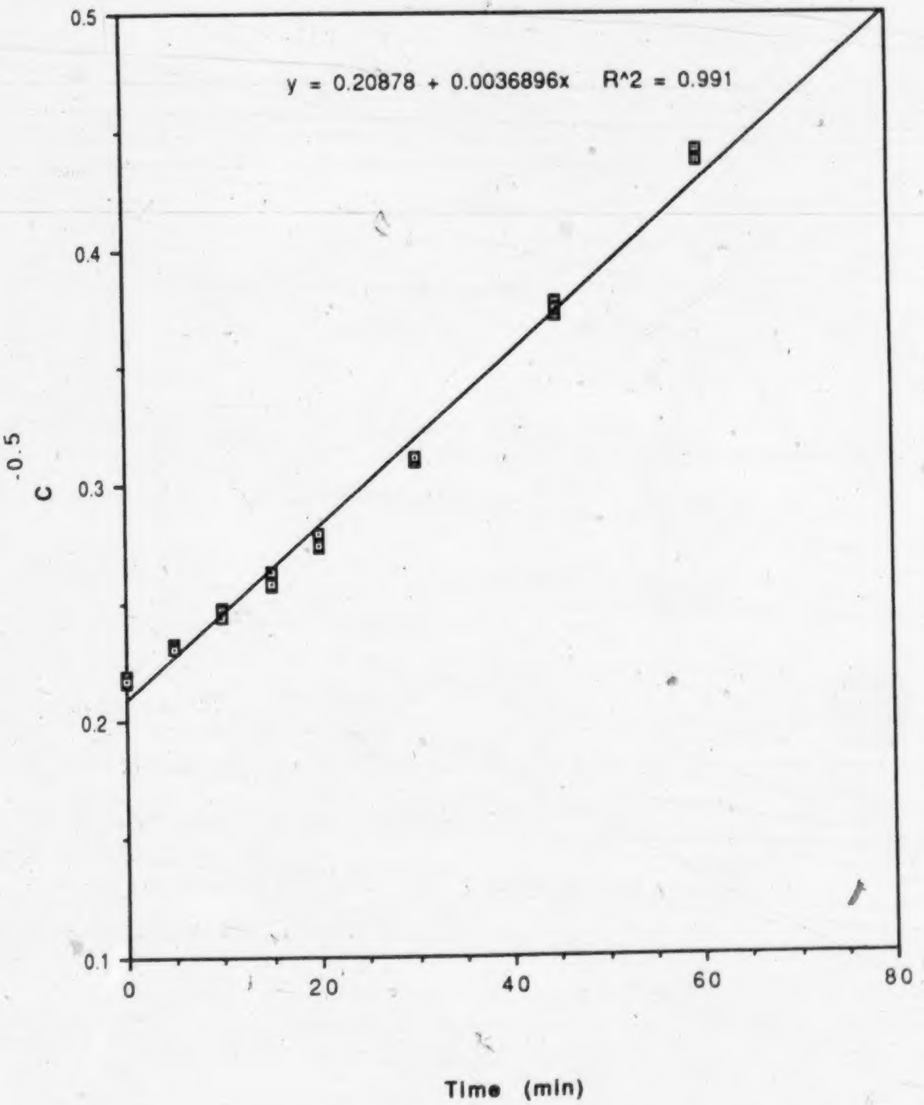


Fig. 4.2 (A-5)

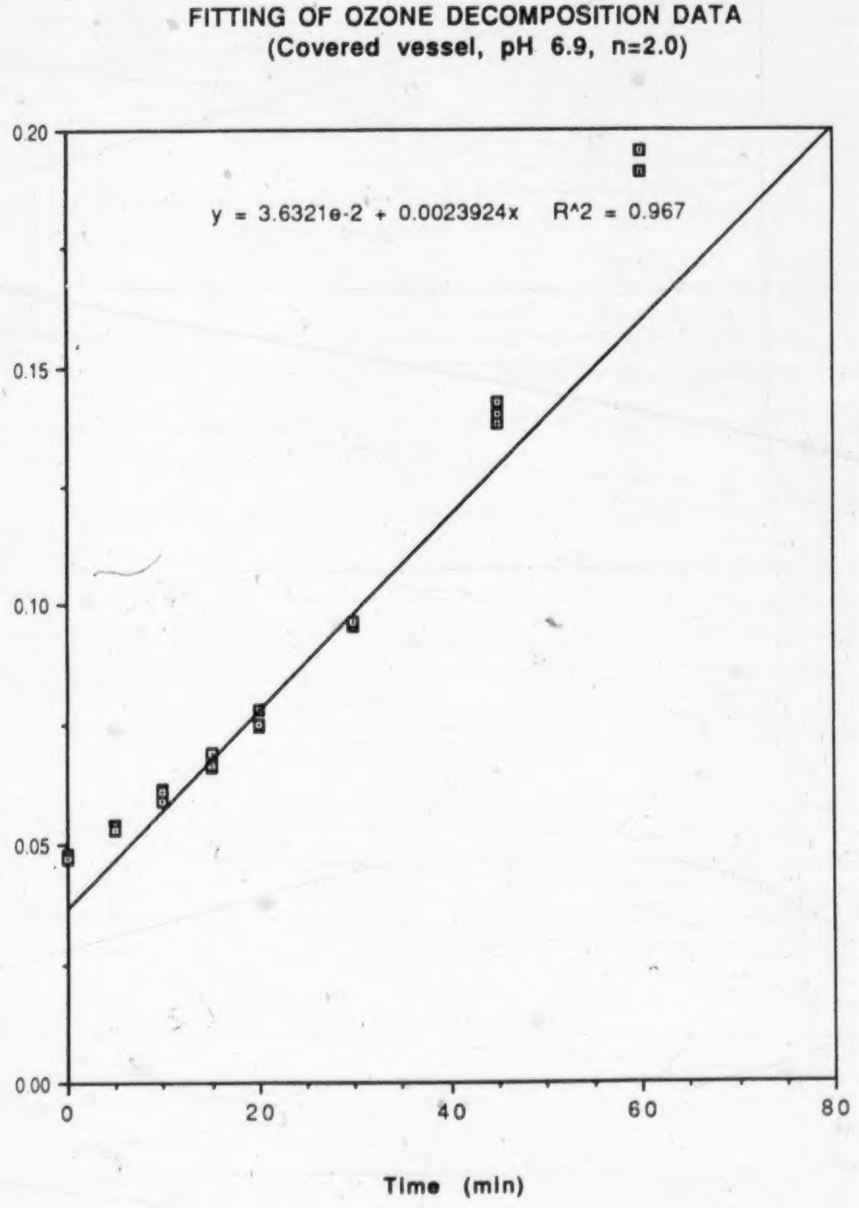

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 6.9, n=2.0)
y = 3.6321e-2 + 0.0023924x R^2 = 0.967
C -1
0.20
0.15
0.10
0.05
0.00
0
20
40
60
80
Time (min)

Fig. 4.2 (B-1)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 6.9, n=0)

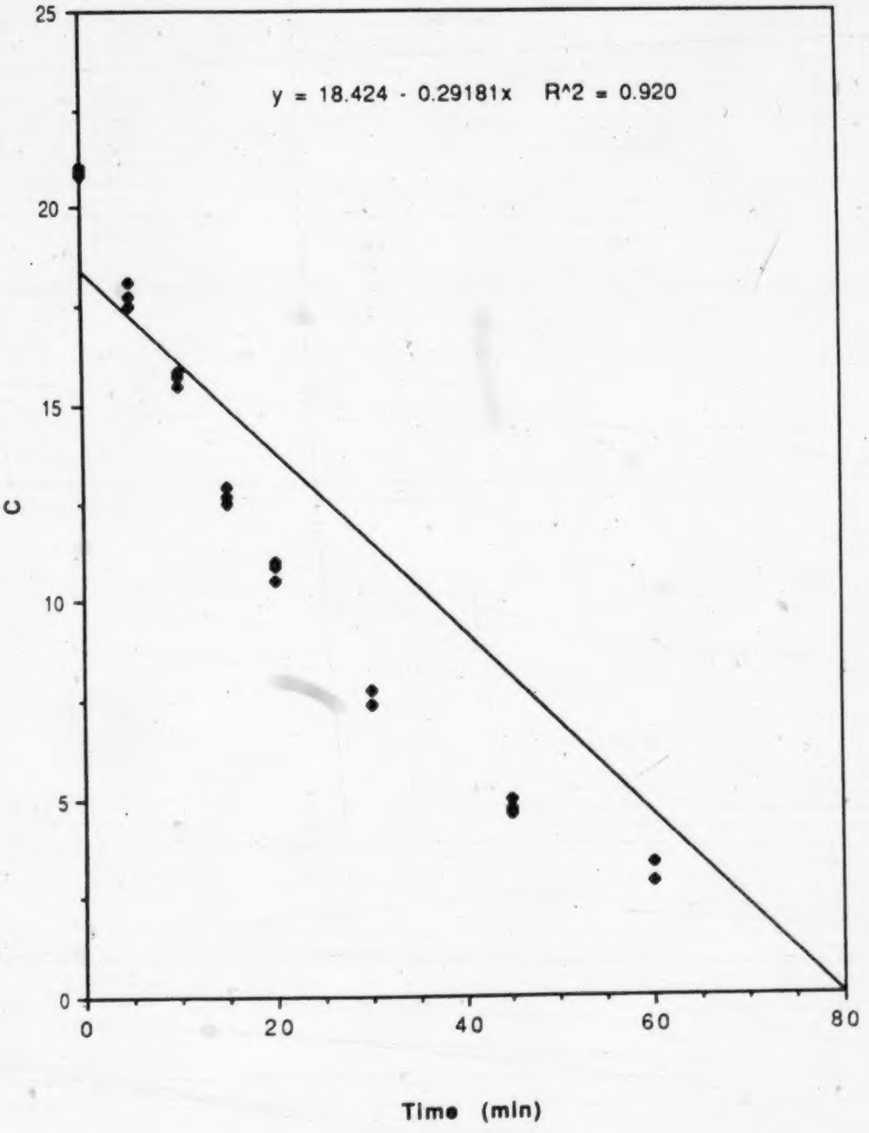


Fig. 4.2 (B-2)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 6.9, n=0.5)

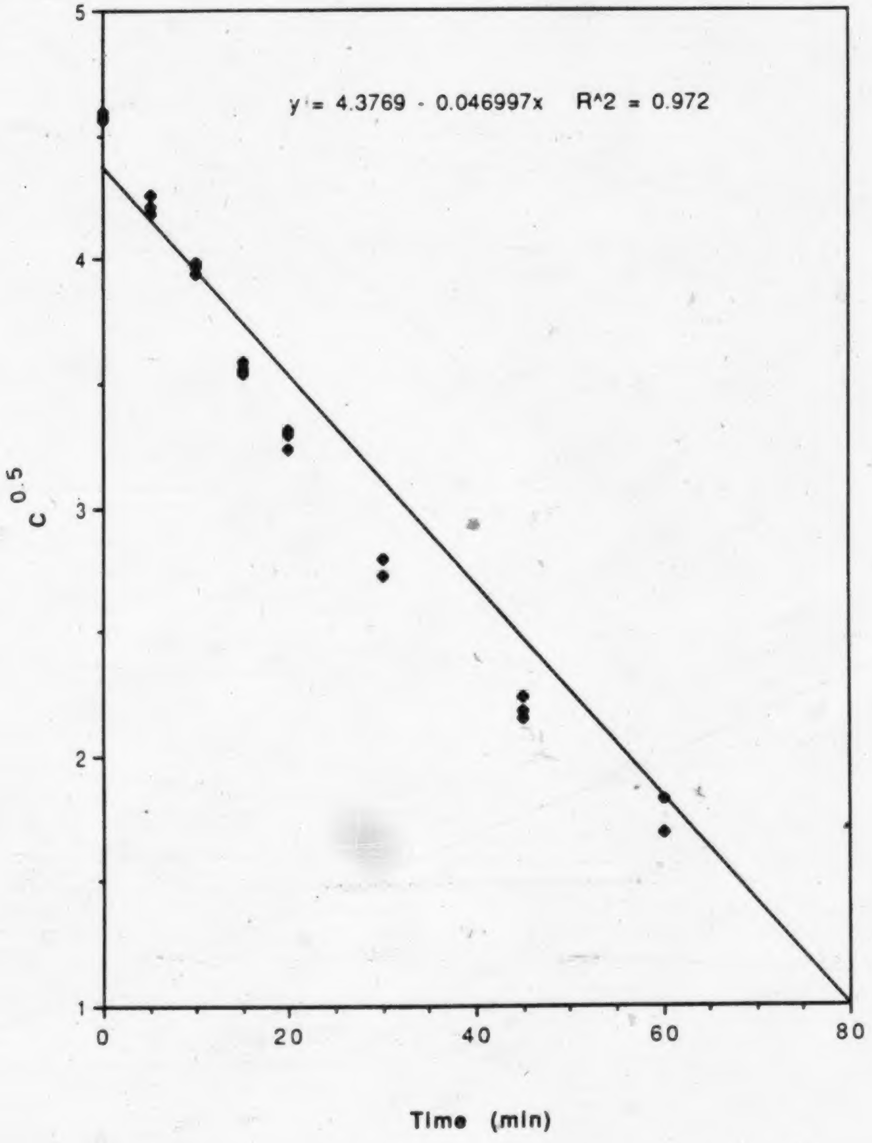


Fig. 4.2 (B-3)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 6.9, n=1.0)

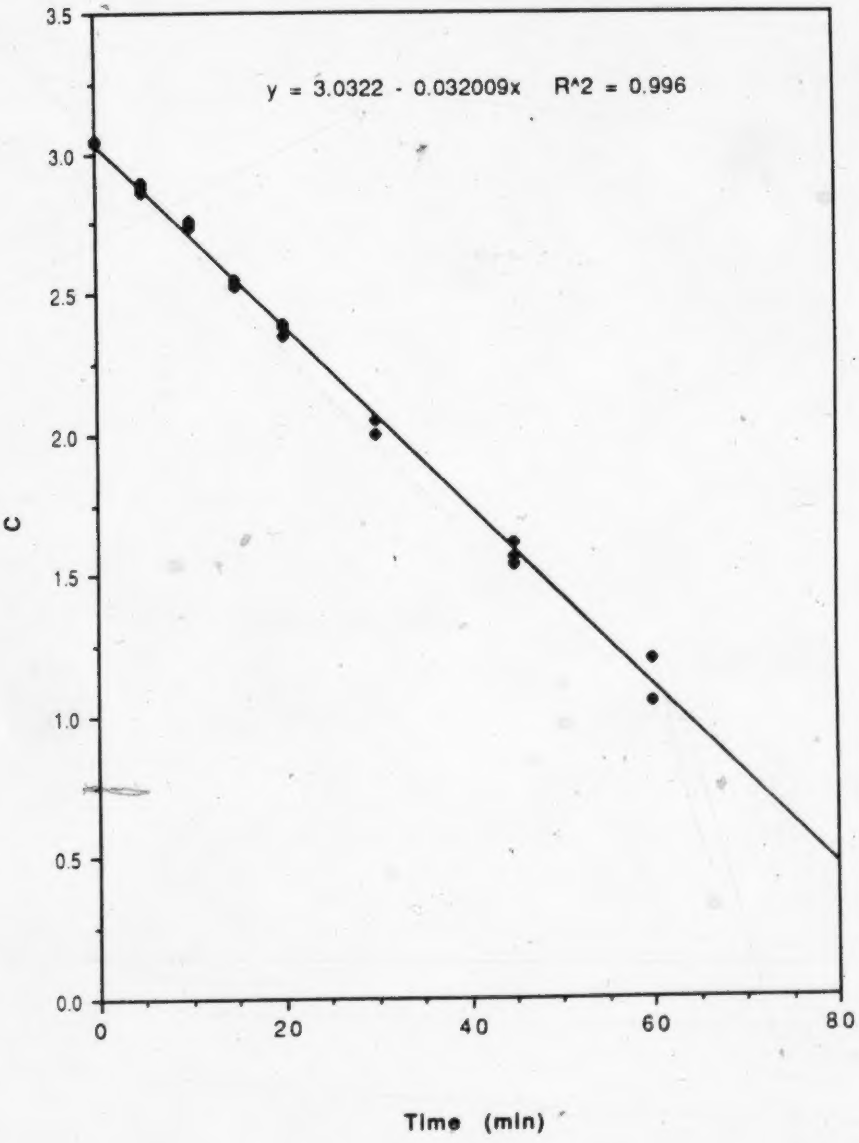


Fig. 4.2 (B-4)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 6.9, n=1.5)

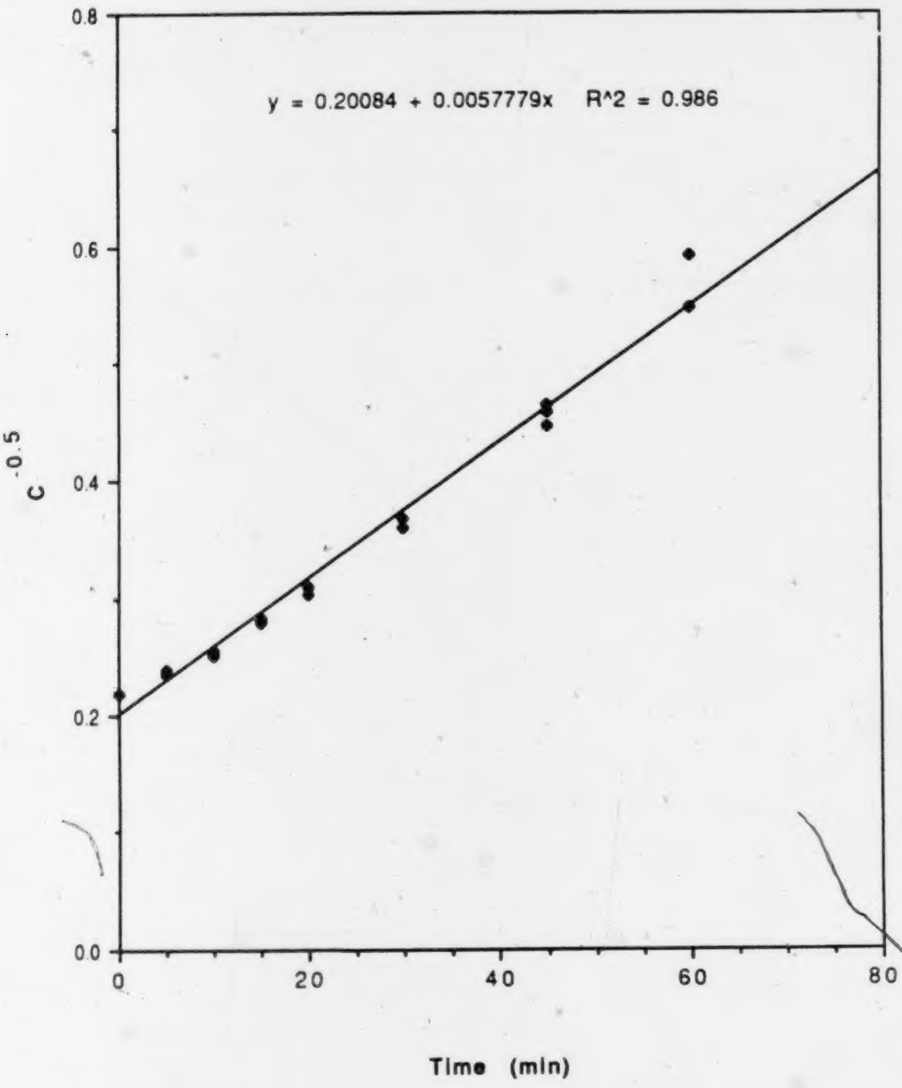


Fig. 4.2 (B-5)

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 6.9, n=2.0)

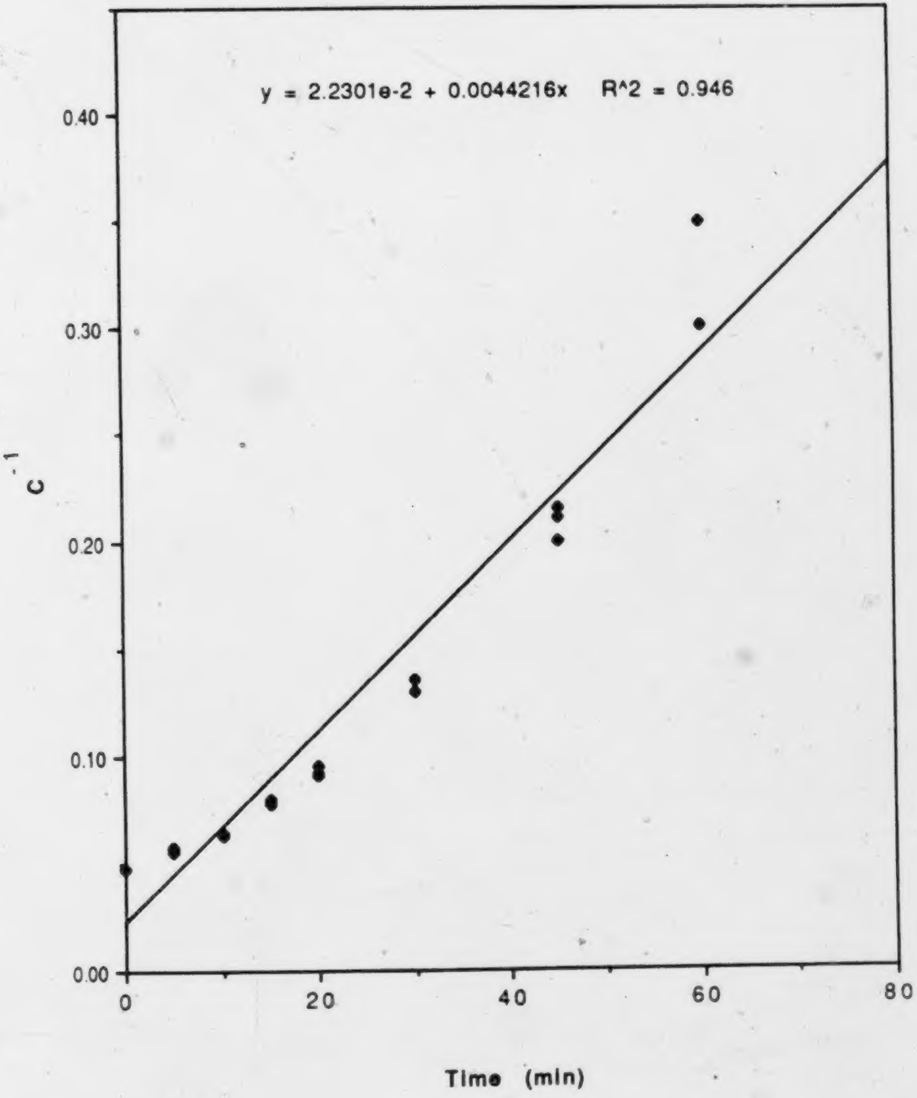


Fig. 4.3 (A-1)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 9, n=0)

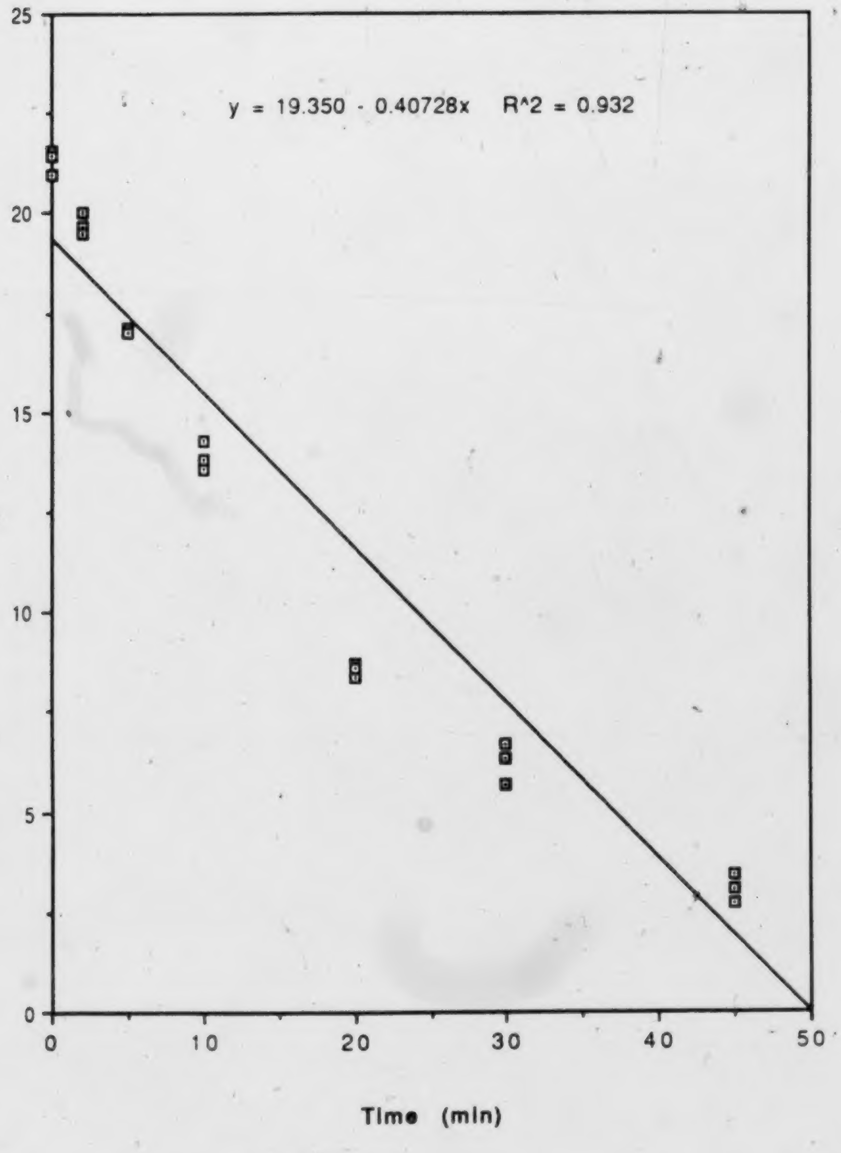


Fig. 4.3 (A-2)

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 9, n=0.5)

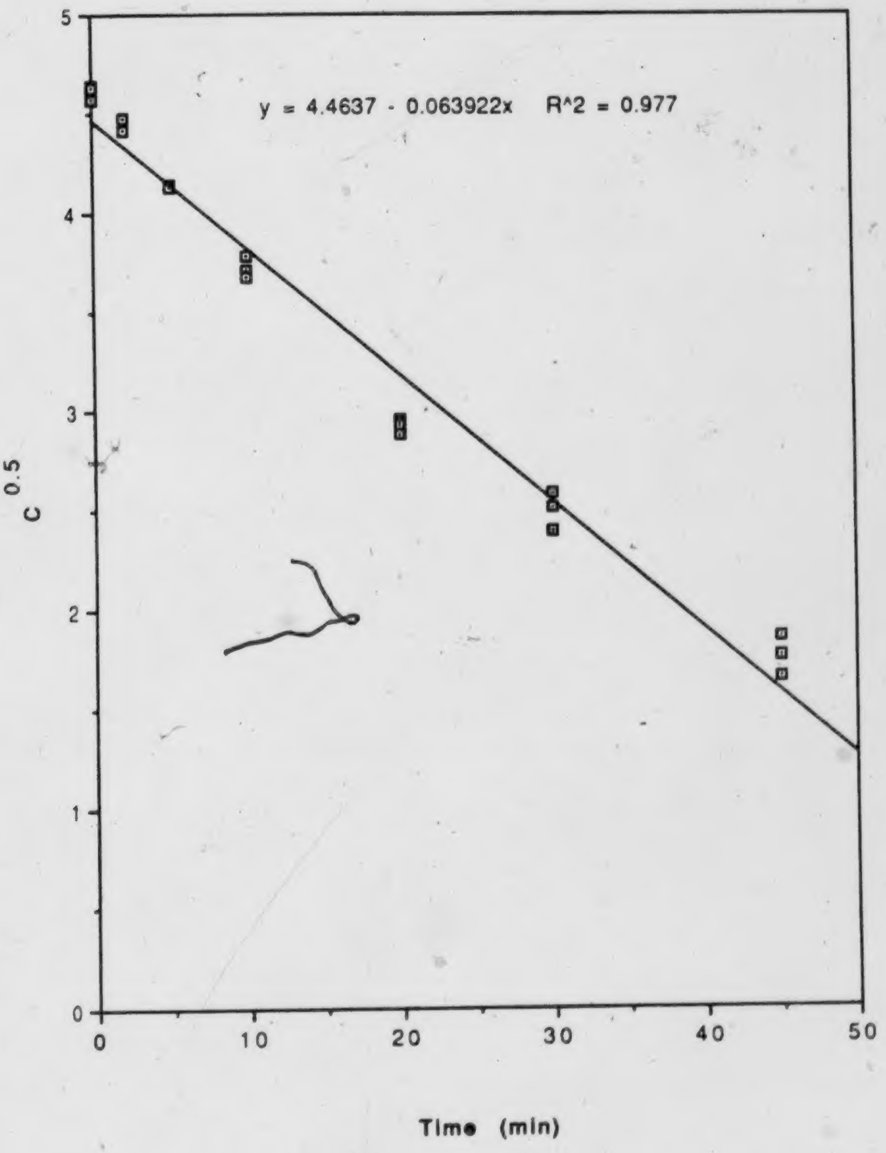


Fig. 4.3 (A-3)

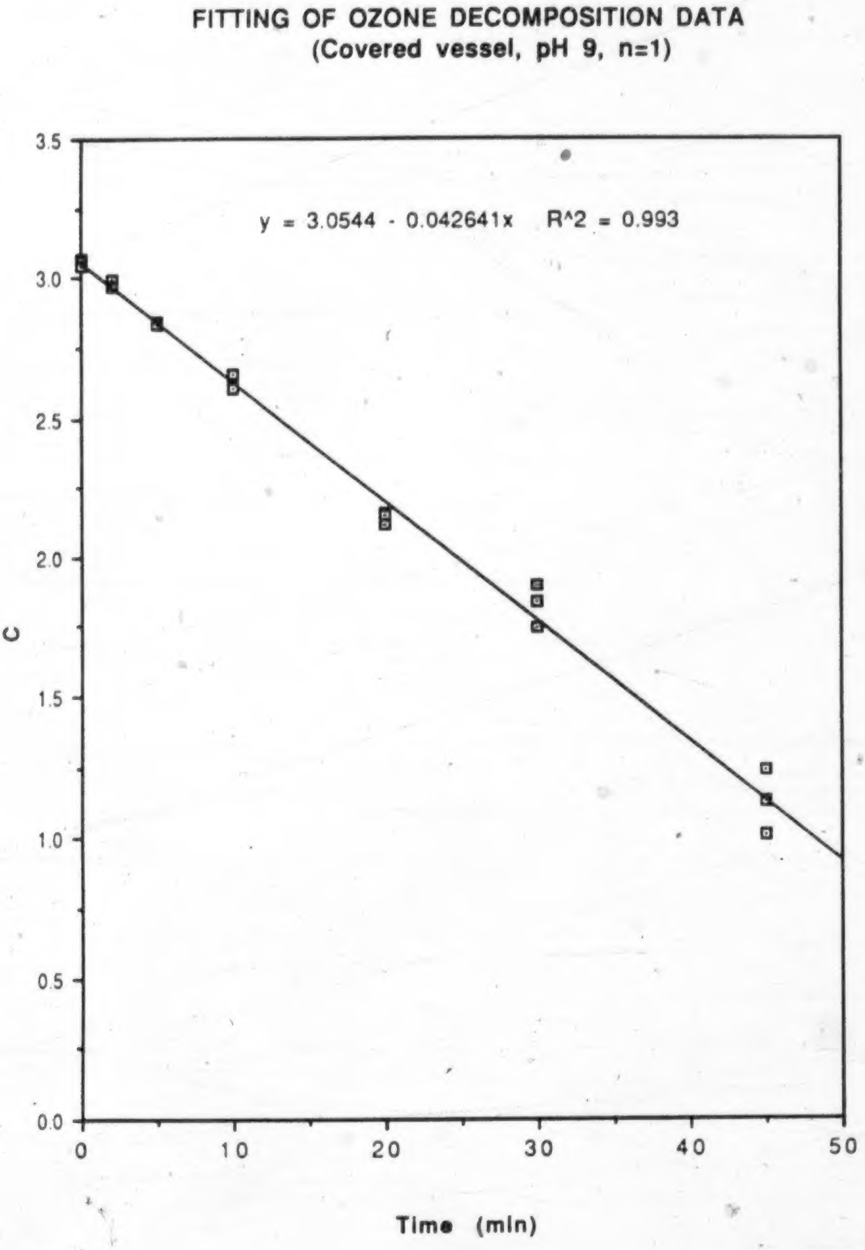

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 9, n=1)
y = 3.0544 - 0.042641x R^2 = 0.993
C
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
Time (min)

Fig. 4.3 (A-4)

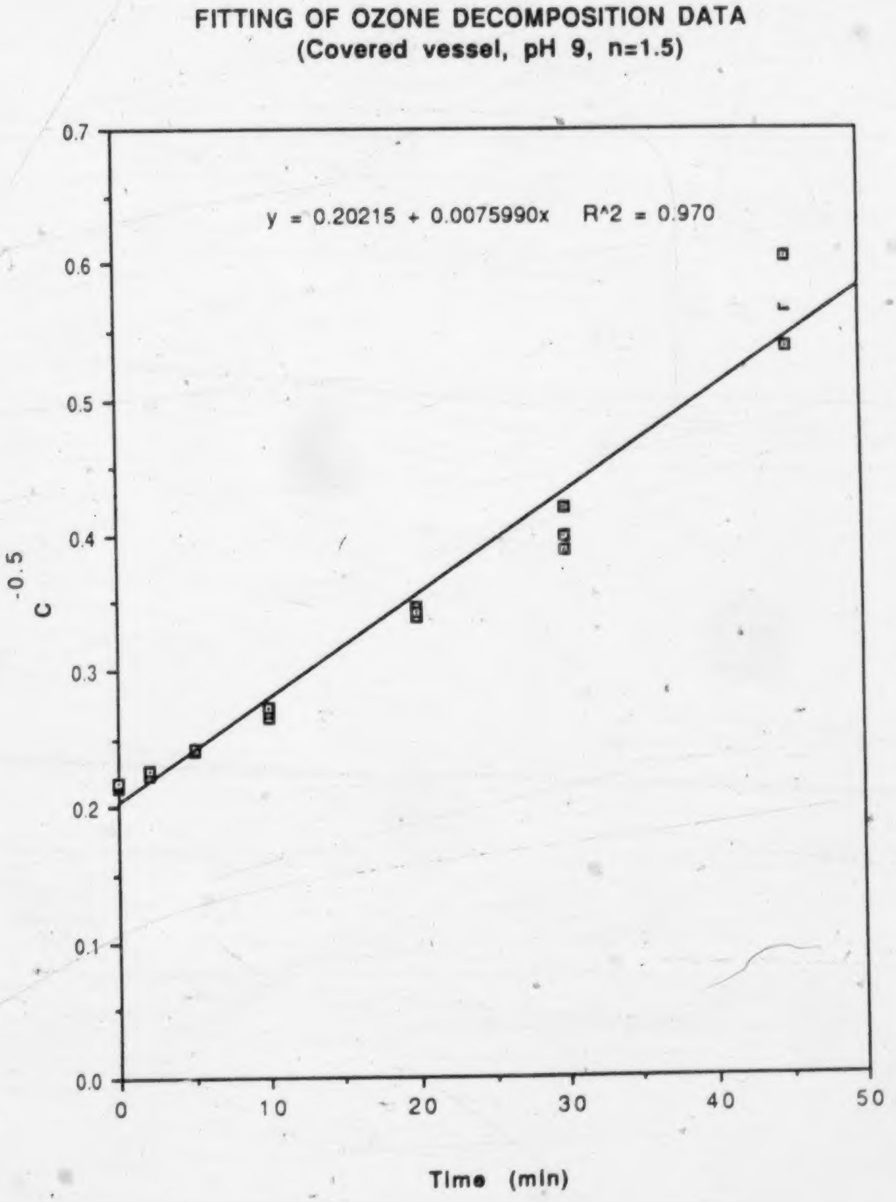

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 9, n=1.5)
y = 0.20215 + 0.0075990x R^2 = 0.970
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
C -0.5
0
10
20
30
40
50
Time (min)

Fig. 4.3 (A-5)

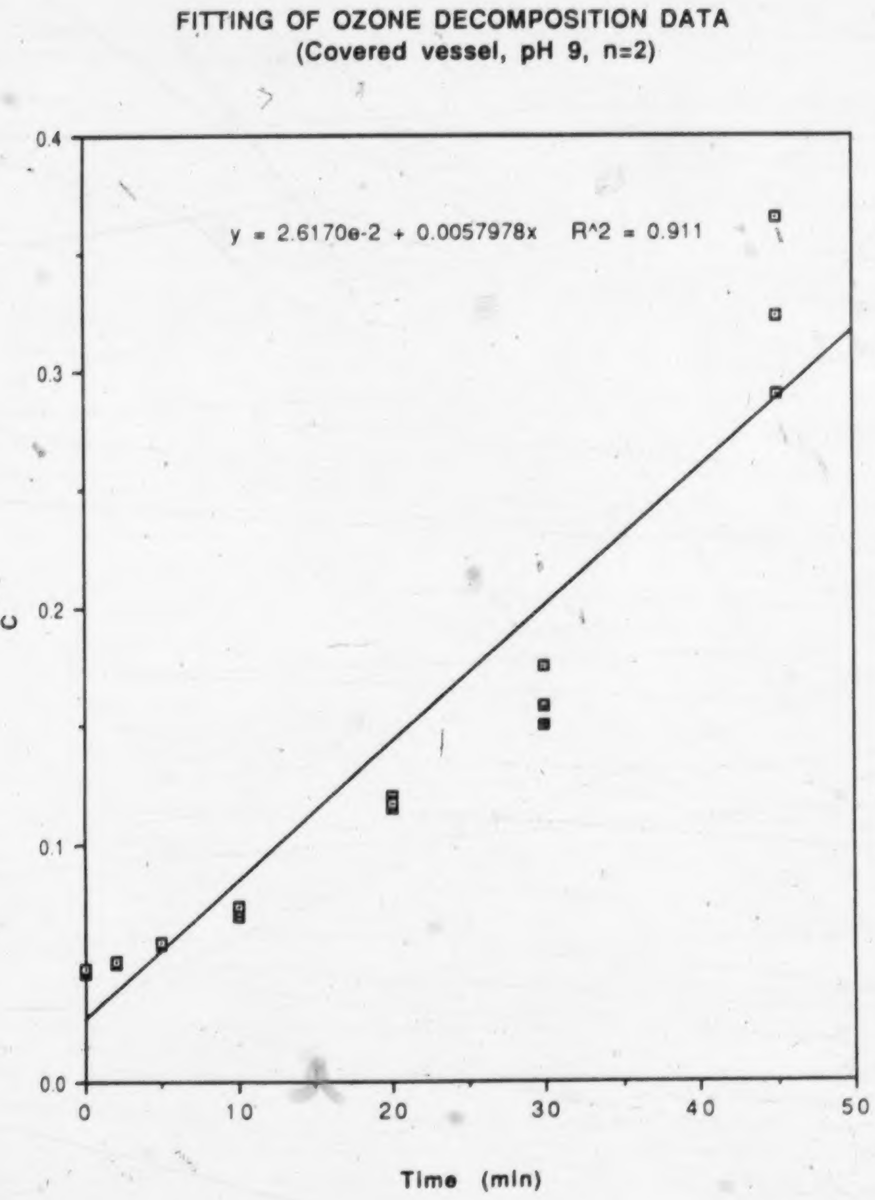

FITTING OF OZONE DECOMPOSITION DATA
(Covered vessel, pH 9, n=2)
y = 2.6170e-2 + 0.0057978x R^2 = 0.911
C -1
0.0
0.1
0.2
0.3
0.4
0
10
20
30
40
50
Time (min)

Fig. 4.3 (B-1)

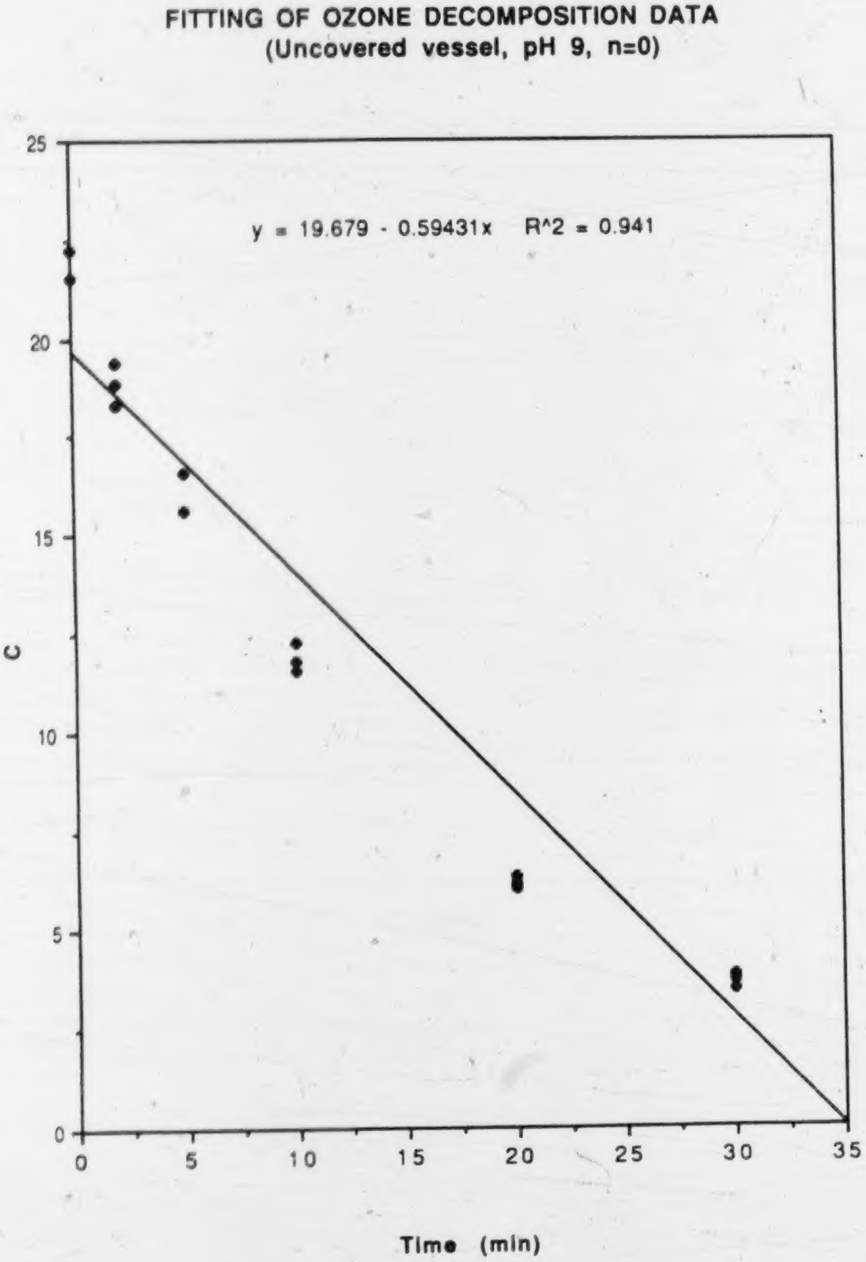

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 9, n=0)
y = 19.679 - 0.59431x R^2 = 0.941
C
Time (min)
0
5
10
15
20
25
0
5
10
15
20
25
30
35

Fig. 4.3 (B-2)

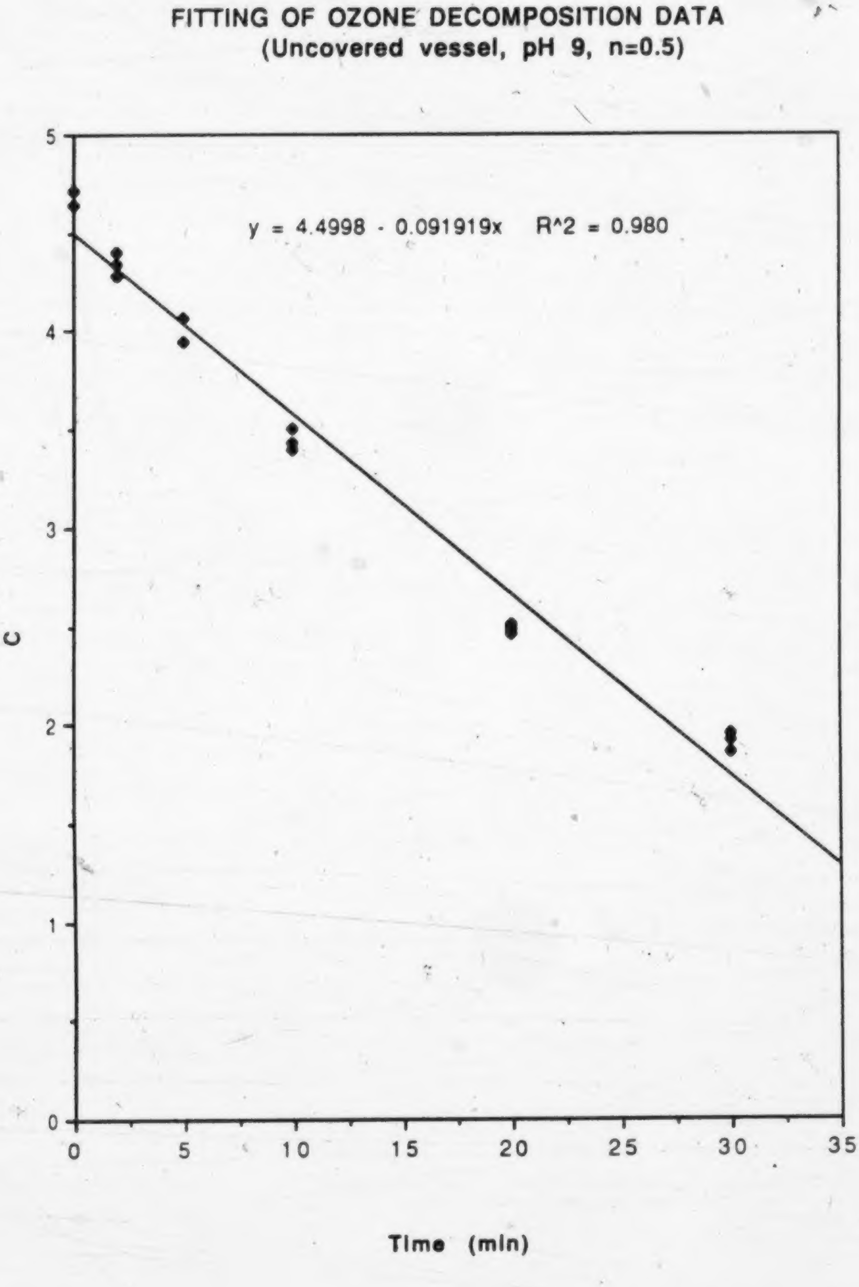

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 9, n=0.5)
y = 4.4998 - 0.091919x R^2 = 0.980
C 0.5
Time (min)
0
1
2
3
4
5
0
5
10
15
20
25
30
35

Fig. 4.3 (B-3)

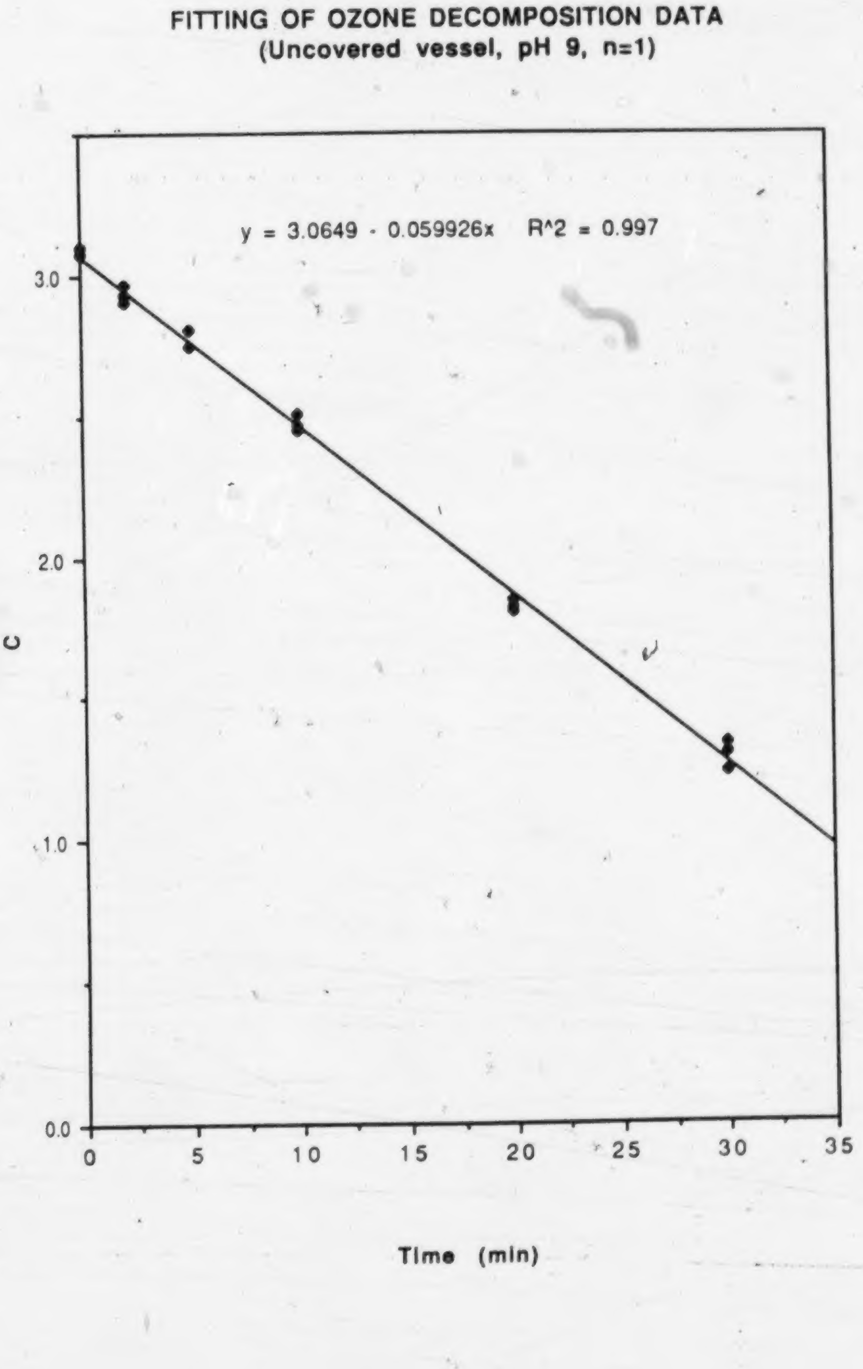

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 9, n=1)
y = 3.0649 - 0.059926x R^2 = 0.997
3.0
2.0
1.0
0.0
C
0
5
10
15
20
25
30
35
Time (min)

Fig. 4.3 (B-4)

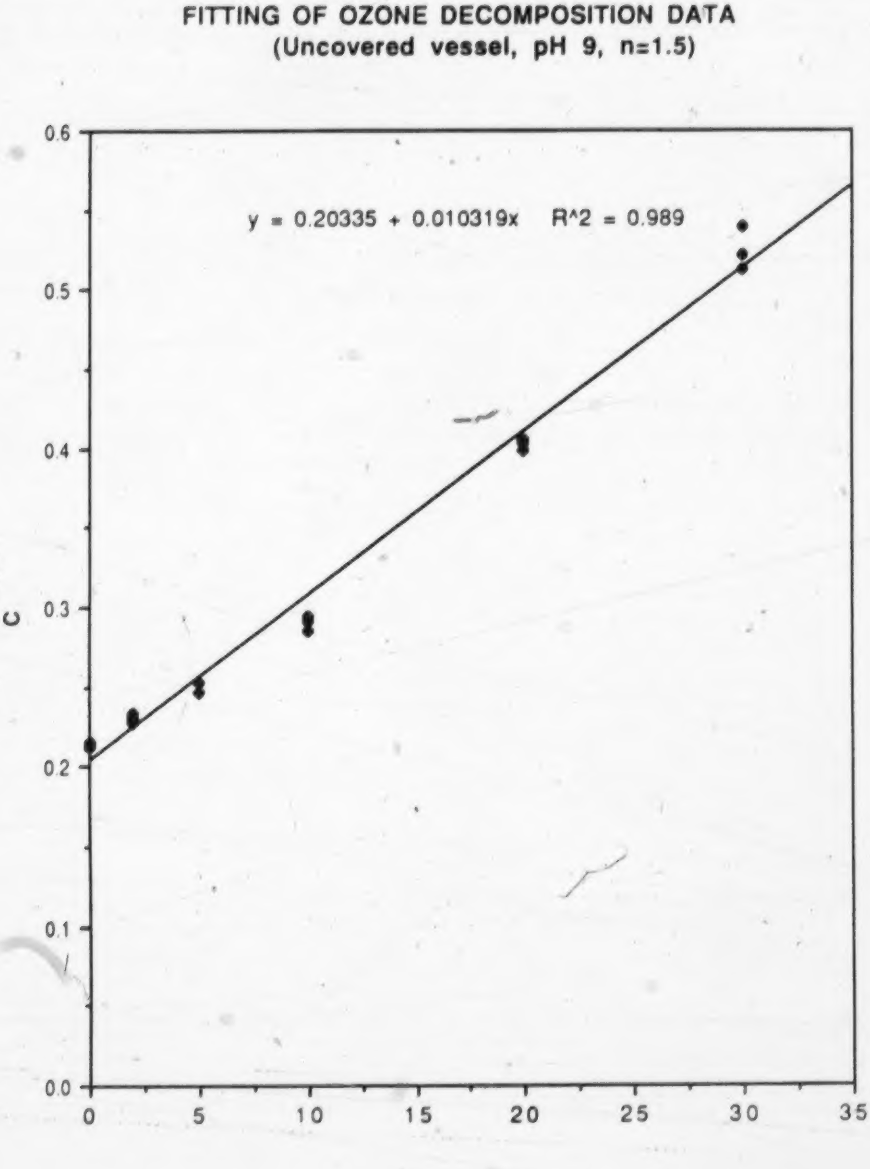
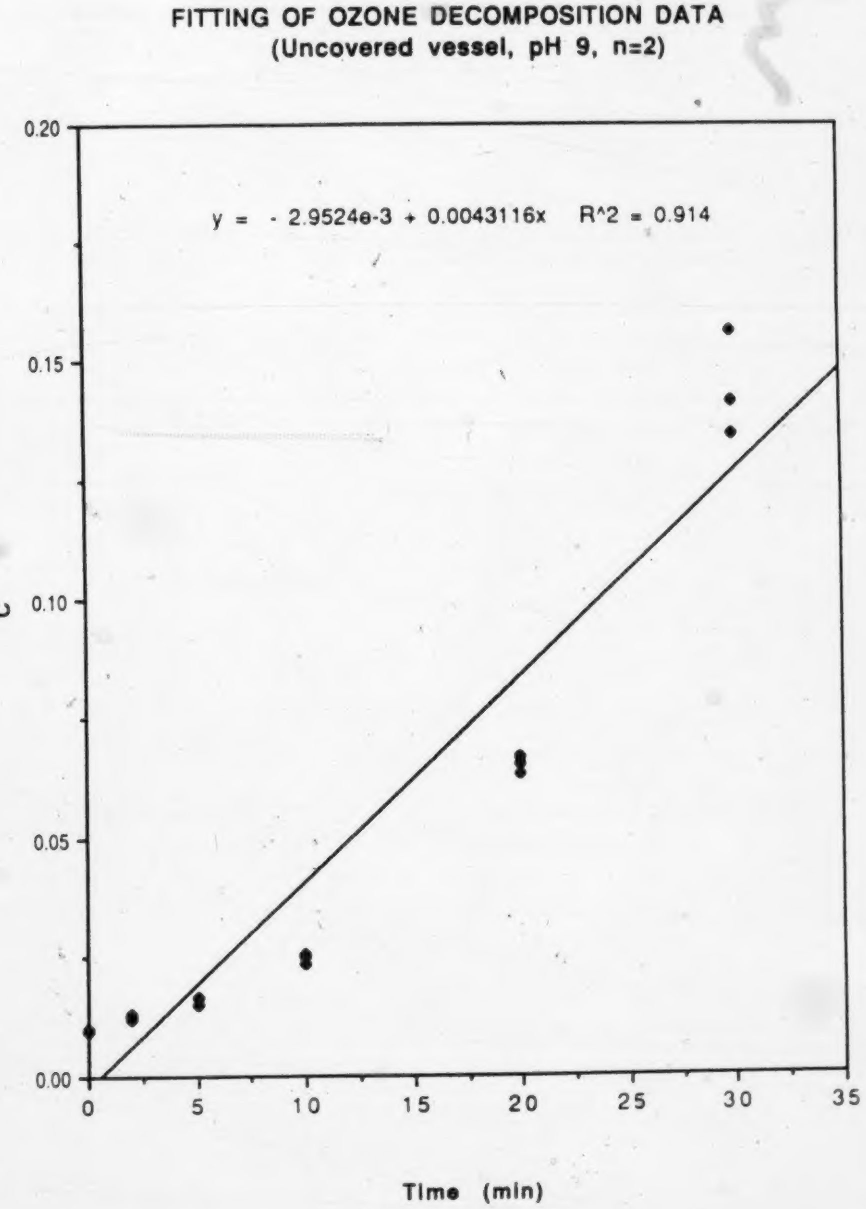

FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 9, n=1.5)
y = 0.20335 + 0.010319x R^2 = 0.989
$C^{-0.5}$
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0
5
10
15
20
25
30
35
Time (min)

Fig. 4.3 (B-5)


FITTING OF OZONE DECOMPOSITION DATA
(Uncovered vessel, pH 9, n=2)
y = - 2.9524e-3 + 0.0043116x R^2 = 0.914
0.20
0.15
0.10
0.05
0.00
C⁻¹
0
5
10
15
20
25
30
35
Time (min)

and uncovered reaction vessels at different pH values used during the experiments (Box *et al.*, 1978). The calculated and values obtained from t-tables are shown in Table 4.1.

Table : 4.1

Comparison of calculated and tabulated t-values

pH	**d.f.**	**t-values**	
		Calculated	**From table at 5% Significance level**
4	33	0.543	1.691
6.9	46	0.991	1.683
9	34	1.171	1.691

The calculated t-values were found not significant at 5% significant level at all the three pH values indicating that during ozone decomposition stripping was not a significant factor in the reduction of ozone concentration from the reaction vessel.

To determine the exact values of ozone half lives and k_1, the reaction rate constant, the first order rate equations were plotted as log (C/C_o) vs. time for pH 4, 6.9 and 9 for covered and uncovered vessels (Figures 4.4 to 4.6). Half lives obtained from these graphs were used to calculate k values using modified form of equ. 4.2 as:

$$\log 0.5 = -k_1 t_{1/2} / 2.303 \qquad \text{(equ. 4.4)}$$

Fig. 4.4

FITTING OF OZONE DECOMPOSITION DATA
(pH 4, n=1)

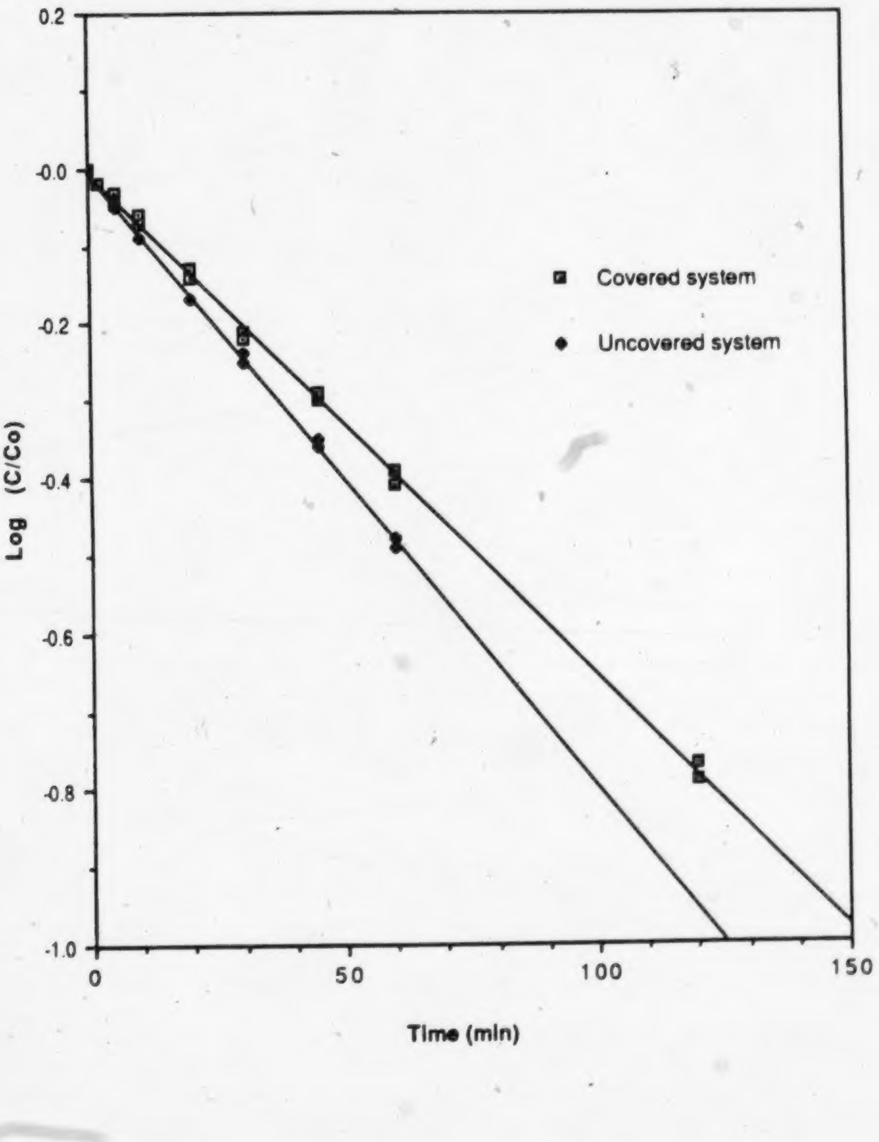


Fig. 4.5

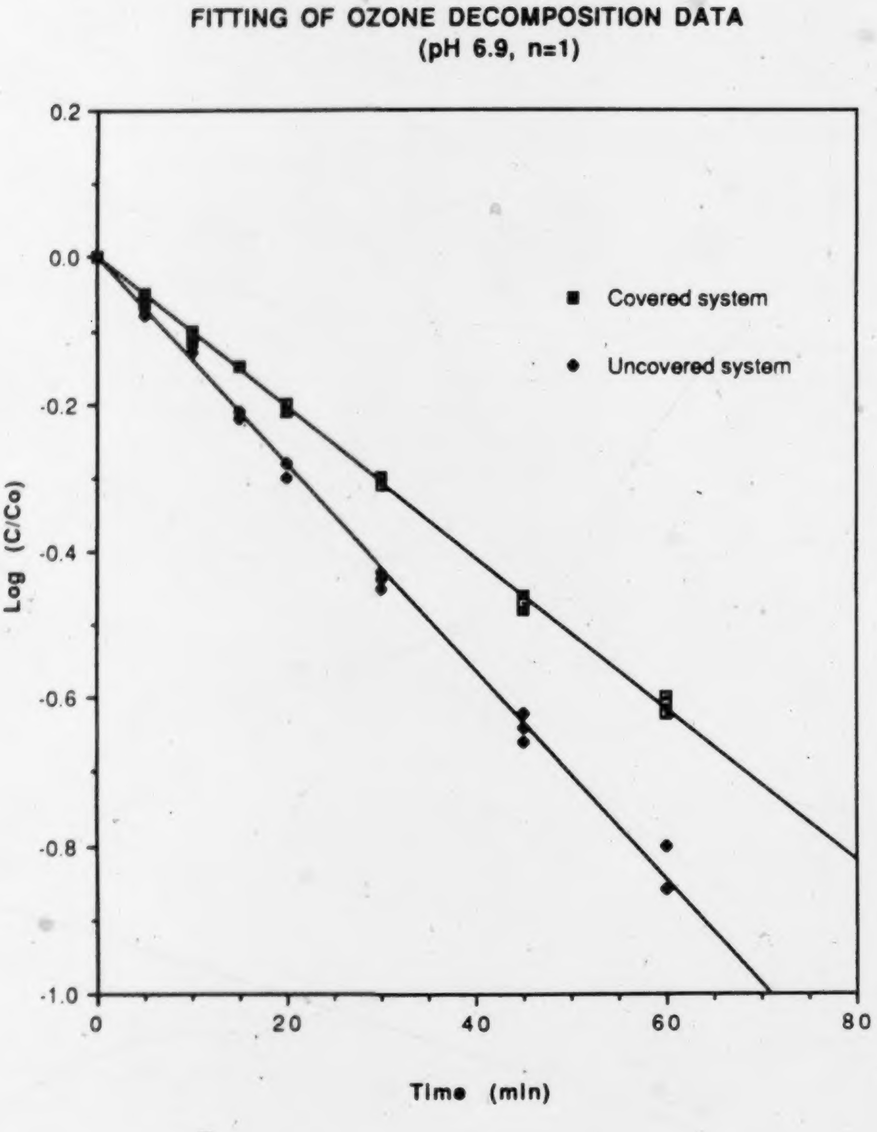
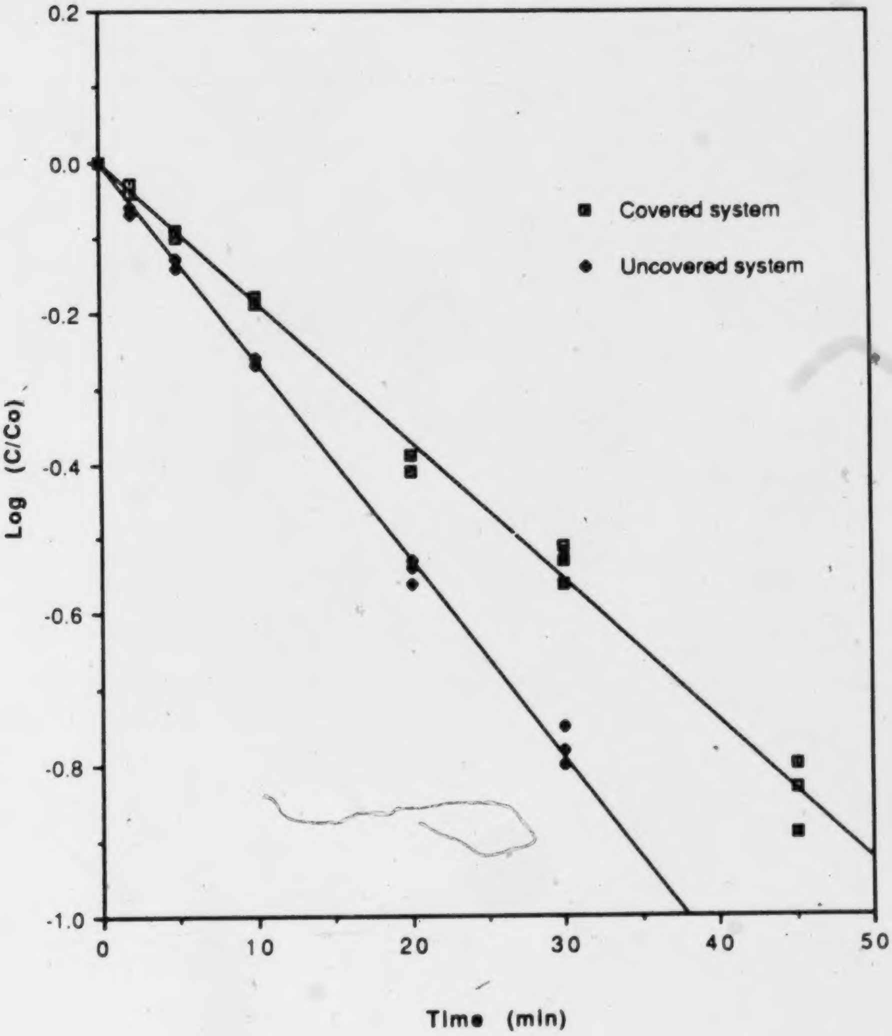

FITTING OF OZONE DECOMPOSITION DATA
(pH 6.9, n=1)
Log (C/Co)
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
0
20
40
60
80
Time (min)
■ Covered system
● Uncovered system

Fig. 4.6

FITTING OF OZONE DECOMPOSITION DATA
(pH 9, n=1)



or $k_1 = 0.693 / t_{1/2}$ (equ. 4.5)

The half lives and k values obtained are mentioned in Table 4.2.

Table : 4.2

Half-lives and observed k values

pH	**Covered vessel**		**uncovered vessel**	
	$t_{1/2}$, min	**k, min^{-1}**	**$t_{1/2}$, min**	**k, min^{-1}**
4	47.0	0.0147	38	0.0182
6.9	26.0	0.0267	18	0.0385
9	17.5	0.0396	12	0.0578

In literature (Table 4.3), the half-lives of ozone varies from 40 sec in 0.05 M phosphate : 0.01 M carbonate buffer solution at pH 10 (Staehelln and Hoigne, 1982) to about 14 h in 0.1 M phosphate : 0.1 M carbonate buffer solution at pH 7.0 to 7.2 (Grunwell *et al.*, 1983). These wide variations in ozone half-lives in aqueous solutions are basically due to the different system conditions of pH, temperature and chemical composition of the solutions used during decomposition studies. In principle, any combination of pH, temperature and reductant that decrease ozone decay rate should result in higher half-lives in aqueous solutions. The studies show that ozone half-life was longer at lower pH and lower temperature. This was because at lower pH, smaller number of hydroxyl ions were available to initiate the decomposition reactions. Similarly,

Table : 4.3

OZONE HALF-LIFE AS A FUNCTION OF pH, TEMPERATURE AND MEDIUM

Medium	pH	Temp. (°C)	Half-life (min)	Reference
Pure water	7.00	-	8.0	Grunwell et al. (1983)
	4.00	25.0	350.0	Hoigne & Bader (1976)
	6.00	25.0	50.0	
	8.00	25.0	3.3	
	10.00	25.0	0.3	
Distilled water	0.65	3.5	115.0	Sullivan & Hill (1980)
	4.85	3.5	81.7	
	7.00	3.5	16.7	
	1.00	20.0	70.0	
	3.00	20.0	50.0	
	0.45	40.0	13.0	
	5.10	40.0	4.2	
	3.50	60.0	4.0	
	5.30	60.0	2.5	
Borate buffer	8.00	20.0	1.8	Gurol & Singer (1982)
	8.00	0.0	120.0	Sennewald (1935)

Medium	pH	Temp. (°C)	Half-life (min)	Reference
Phosphate buffer	5.29	0.0	78.3	Sennewald (1935)
	6.00	0.0	63.3	
	7.00	0.0	41.7	
	8.00	0.0	120.0	
0.1 M phosphate buffer	7.00 -7.20	-	43.3	Grunwell et al. (1983)
0.25 M phosphate buffer	7.00 - 7.20	-	75.0	
0.01 M carbonate buffer	7.00 - 7.20	-	750.0	Grunwell et al. (1983)
0.1 M carbonate buffer	7.00 - 7.20	-	683.0	
0.002 M bicarbonate buffer	6.00	25.0	166.7	Hoigne & Bader (1976)
	8.00	25.0	2.3	
	10.00	25.0	1.0	
0.1 M phos., 0.01 M carb.	7.00 - 7.20	-	666.7	Grunwell et al. (1983)
0.1 M phos., 0.1 M carb.	7.00 - 7.20	-	833.3	
0.25 M phos., 0.01 M carb.	7.00 - 7.20	-	283.3	
0.25 M phos., 0.1 M carb.	7.00 - 7.20	-	316.7	
0.05 M phos., 0.01 M carb.	10.00	-	0.7	Staehelln & Hoigne(1982)
HClO4	2.20	0.0	110.0	Alder & Hill (1950)
	2.15	0.0	158.3	
	1.30	27.0	55.0	
	1.00	27.5	550.0	

Medium	pH	Temp. (°C)	Half-life (min)	Reference
Sulfuric acid	2.00	0.0	4833.3	Weiss (1935)
	3.70	0.0	2166.7	
	4.00	0.0	2000.0	
0.05 M phosphate buffer	4.00	21.0	46.7	This study (covered vessel with stirring)
	6.90	21.0	26.0	
	9.00	21.0	18.3	
	4.00	21.0	38.3	This study (uncovered vessel with stirring)
	6.90	21.0	18.0	
	9.00	21.0	12.0	

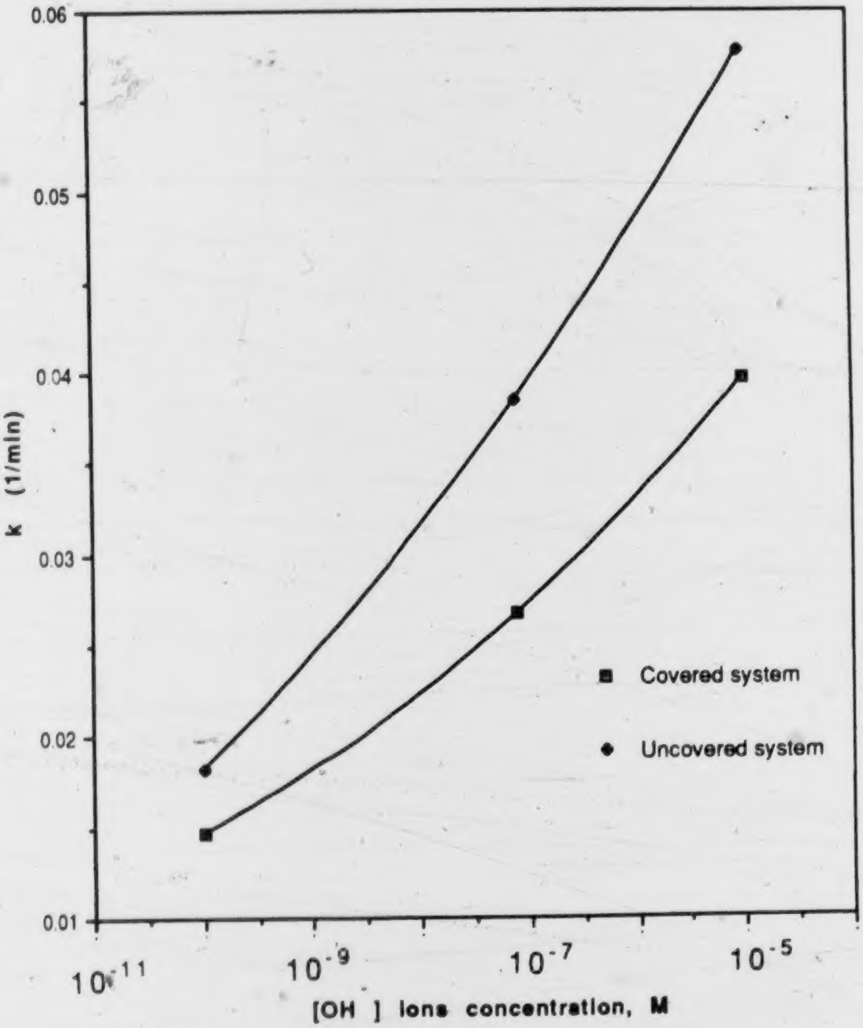
increase in temperature increases the instability of ozone molecules and activates its decomposition. The solution composition exerts a marked influence on the rate of decomposition since the impurities (e.g. carbohydrates, methanol etc.) present in the medium may act as promotors in radical type chain reactions and thus result in increased rate of ozone decomposition. On the other hand, phosphate, bicarbonate, carbonate as well as some special organic compounds act as hydroxyl radicals scavengers and thus increase the ozone half life. It can be, therefore, said that in good ground waters, containing bicarbonate and carbonate ions, the half-life of ozone may be longer than in the distilled water of comparable pH (Hoigne, 1988).

In the presence of high concentrations of radical scavengers, a linear relationship can be obtained between reaction rate constant and OH^- ions of the solution (Staehelln and Hoigne, 1982). In the present study this was not the case (Figure 4.7) which can be attributed to the lower scavengers concentration. It was, however, observed that reaction rate was influenced by the pH variations in the reaction system and was in agreement with the literature (Gurol and Singer, 1982; Hoigne, 1988).

It is worth mentioning that all the ozone decomposition studies available in literature were conducted with the aim to maximize the ozone stability and thus did not involve any stirring of the solutions. In the present case, on the other hand, the basic objective was to study the decomposition rate to be utilized in subsequent ozone dose - bacterial response experiments, where stirring was a basic requirement to provide equal chances of contact

Fig. 4.7

OBSERVED FIRST ORDER RATE CONSTANTS OF OZONE DECOMPOSITION AS A FUNCTION OF [OH^-] IONS CONCENTRATION



between ozone molecules and organism; therefore, stirring was involved in the decomposition studies. Further, bicarbonate and carbonate ions are more powerful OH° radicals scavengers than phosphate ions, thus more effective in terminating the ozone decomposition chain reactions.

In any reaction equation, the reaction rate constant, k, is a function of temperature and pH of the system and can be related to these parameters by:

$$k = k_o \{ \exp [-E/R (T_2-T_1)]\} [OH^-]^b$$

Where,

k_o = apparent frequency factor, (mole O_3/L)$^{1-n}$ (mole OH^-/L)$^{-b}$ (min)$^{-b}$
and for n = 1 it is reduced to (mole OH^-/L)$^{-b}$ (min)$^{-1}$,
E = Arrhenius activation energy in cal/mole,
R = ideal gas law constant, (1.987 cal/mole °K),
T = absolute temperature, °K,
OH^- = hydroxide ion concentration, mole OH^-/L,
b = constant.

Because, the temperature was kept constant in the present study, therefore, k is a function of OH^- ions concentration and is reduced to:

$$k = k_o [OH^-]^b \qquad \text{(equ. 4.6)}$$

Since OH^- is a function of pH of the solution and can be calculated by the following relationship:

$$pH + pOH = 14$$

where,

$$pH = -\log [\, H^+], \text{ and}$$

$$pOH = -\log [\, OH^-]$$

Thus for pH 4, 6.9 and 9; the respective $[\, OH^-]$ ion concentrations would be 10^{-10}, $10^{-7.1}$ and 10^{-5} moles/L. The values of k and b were determined by linear regression (Figure 4.8) after transforming the equ. 4.6 into log form as follow:

$$\log k = \log k_o + b \log [\, OH^-]$$

The resulting equations were as follow,

For covered vessel:

$$\log k = -0.965 + .086 \log [\, OH^-]$$

or $$k = 0.108\, [\, OH^-]^{.086} \qquad \text{(equ. 4.7)}$$

For uncovered vessel:

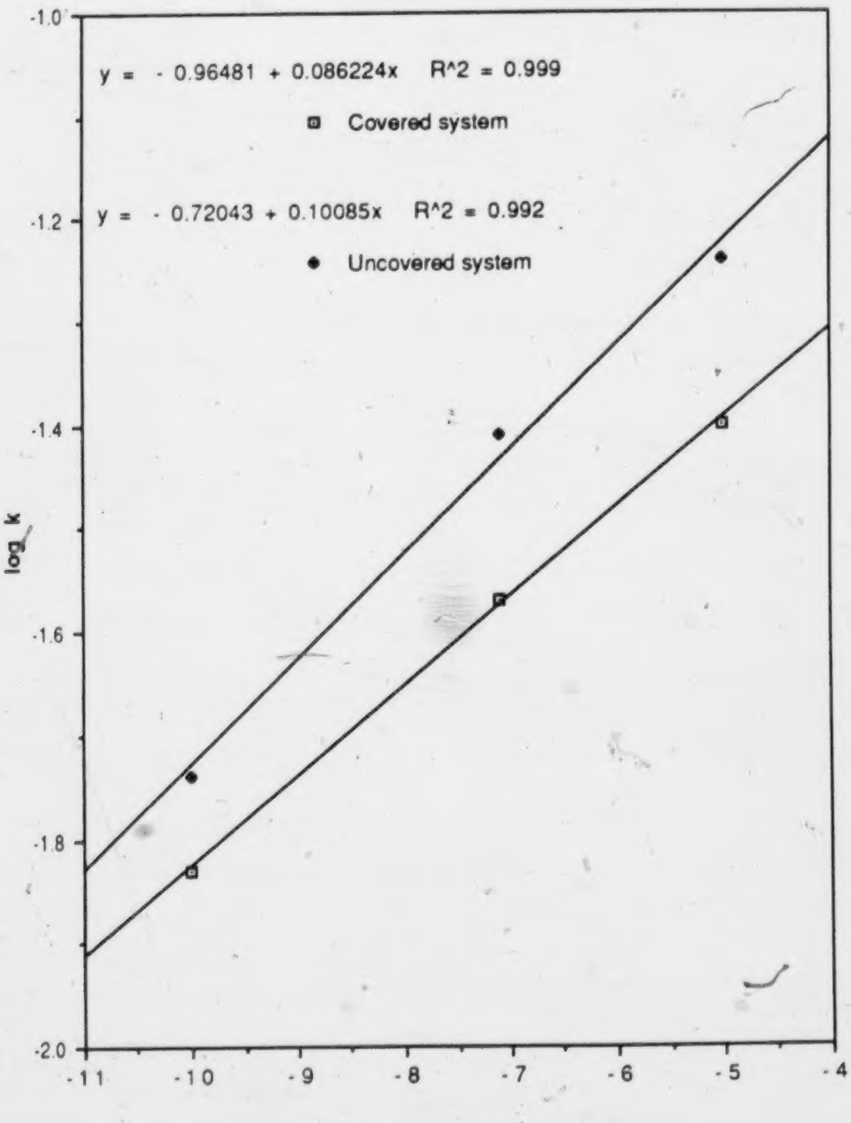
$$\log k = -0.720 + 0.101 \log [\, OH^-]$$

or $$k = 0.191\, [\, OH^-]^{.101} \qquad \text{(equ. 4.8)}$$

The squares of overall correlation coefficients, R^2, for these equations were 0.999 and 0.992, respectively. The values of b obtained are much smaller than those reported in literature. Sullivan

Fig. 4.8

LOG k VS. LOG [OH] IONS CONCENTRATION



log [OH] ions concentration

and Roth (1980), Gurol and Singer (1982; the decomposition was second order in this case) and Sugimitsu *et al.* (1989) obtained b values as 0.123, 0.55 and 0.7, respectively. These equations were used to calculate k values and are reported in Table 4.4.

Table : 4.4

k values for covered and uncovered reaction vessels

pH	**Calculated k values, (1/min)**	
	Covered vessel	**Uncovered vessel**
4	0.0149	0.0187
6.9	0.0265	0.0366
9	0.0401	0.0597

Substituting k relationships in to the first order decomposition equation yielded:

For covered vessel,

$$- dO_3/dt = 0.108\ [\ OH^-\ ]^{.086}\ [O_3] \qquad \text{(equ. 4.9)}$$

For uncovered vessel,

$$- dO_3/dt = 0.191\ [\ OH^-\ ]^{.101}\ [O_3] \qquad \text{(equ. 4.10)}$$

The ozone concentrations were determined using these developed equations and comparison with observed values was found not significant at 5% level.

Only one study (Czapski *et al.*, 1968) was found in which ozone decomposition was studied in an alkaline medium by varying the pH from 10 to 13 at a constant temperature of 25°C using a stopped flow spectrophotometric technique. Czapski *et al.* found that the ozone decay followed first order kinetics and the k value obtained was 700 M^{-1} sec^{-1}.

In summary, it was concluded for a time period of 120 sec that the stripping of ozone from the reaction vessel was not a significant factor in ozone decomposition studies in 0.05 M phosphate buffer solution at a pH range of 4 to 9. The ozone decomposition followed a first order kinetics and no change in its mechanism was observed in this pH range. The reaction rate constant was found proportional to the pH of the solution. It suggested that the initiation of the ozone decay was due to the presence of OH^- ions. Other studies suggest that the subsequent chain reactions depend upon the relative amounts of promotors and scavengers present in the system.

4.2 EFFECTS OF pH ON BACTERIAL SURVIVAL:

To observe the effects of pH variations on the bacterial survival, 20 independent observations were taken at pH 4, 6.9 and 9 using 0.05 M phosphate buffer solution in covered and uncovered vessels. The samples were drawn at 0, 15, 30, 45 and 60 min. The survival of the bacterial populations with respect to time is summarized in Table 4.5, and the data is plotted in Figures 4.9 to 4.11 in the form of log number of survival (log N/dL) and the ratio of survival (N/N_0) vs. time for different pH values used in the experiments. The raw data is given in appendix 3.

It has been mentioned in the literature that factors such as pH, nature of buffer solutions, presence of sodium chloride and cations may affect the survival of microorganisms as well as the disinfection process itself. These effects may be due to the changes in the chemistry of the disinfectant on one hand and in the characteristics of the bacterial cell on the other (Morris, 1970). The effects of pH variations on the chemistry of disinfectants have already been discussed in section 1.2. With regard to the effects associated with the pH changes on the cell, it is anticipated that these alter the ionic charge and interfacial potential at the microbial surface with consequent effect on the rate of cell growth (Morris, 1970). Russel (1982) pointed out that increase in the pH of the medium results in the increase of number of negatively charged groups on the surface of the bacteria, so the positively charged disinfectant molecules have an enhanced degree of binding with the negatively charged bacteria. Hannan (1953) stated that

> "under extreme pH conditions, the adverse effects appear to be on the cell nucleus to produce a high frequency of mutation, a proportion of which leads to a failure to reproduce".

He further commented that

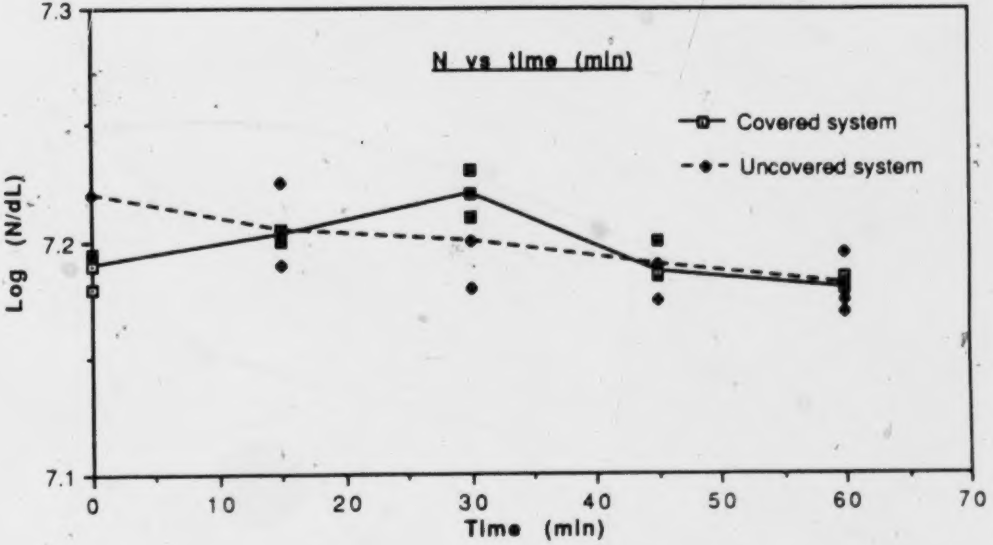
Table: 4.5

EFFECTS OF pH VARIATIONS ON BACTERIAL SURVIVAL
(No - 10^7.20 CFU/dL)

No.	Time (min)	Covered system			Uncovered system		
		pH 4	pH 6.9	pH 9	pH 4	pH 6.9	pH 9
1	0	7.18 - 7.20	7.20	7.19 - 7.22	7.22	7.19 - 7.22	7.18 - 7.23
2	15	7.20 - 7.21	7.19 - 7.23	7.18 - 7.22	7.18 - 7.24	7.20 - 7.22	7.16 - 7.23
3	30	7.20 - 7.26	7.18 - 7.20	7.20	7.18 - 7.22	7.18 - 7.20	7.16 - 7.23
4	45	7.17 - 7.20	7.17 - 7.19	7.18 - 7.19	7.18 - 7.20	7.16 - 7.20	7.16 - 7.18
5	60	7.17 - 7.19	7.13 - 7.16	7.16 - 7.20	7.17 - 7.20	7.15 - 7.18	7.17 - 7.20

Fig. 4.9

BACTERIAL SURVIVAL IN 0.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^7.2 CFU/dL, pH 4)



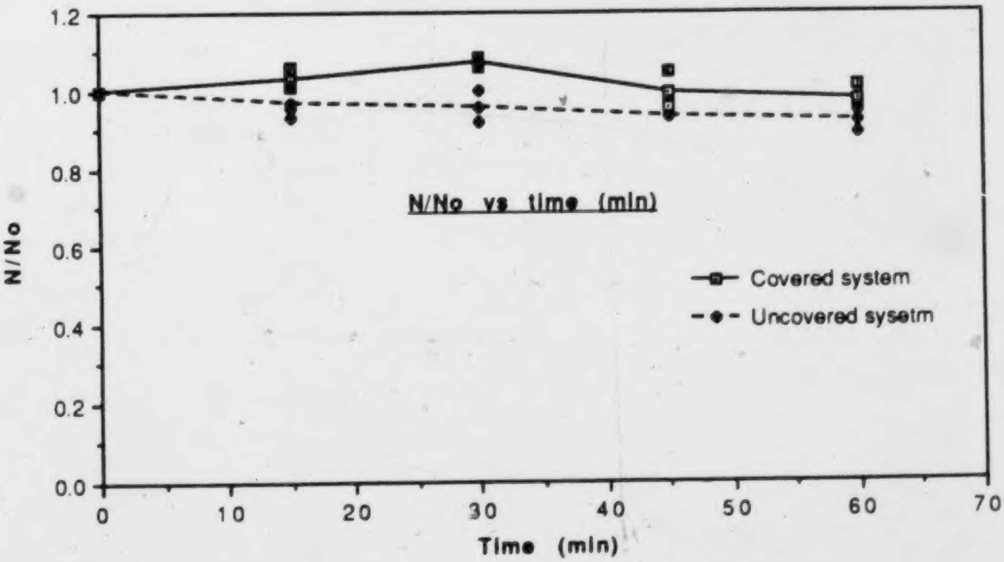
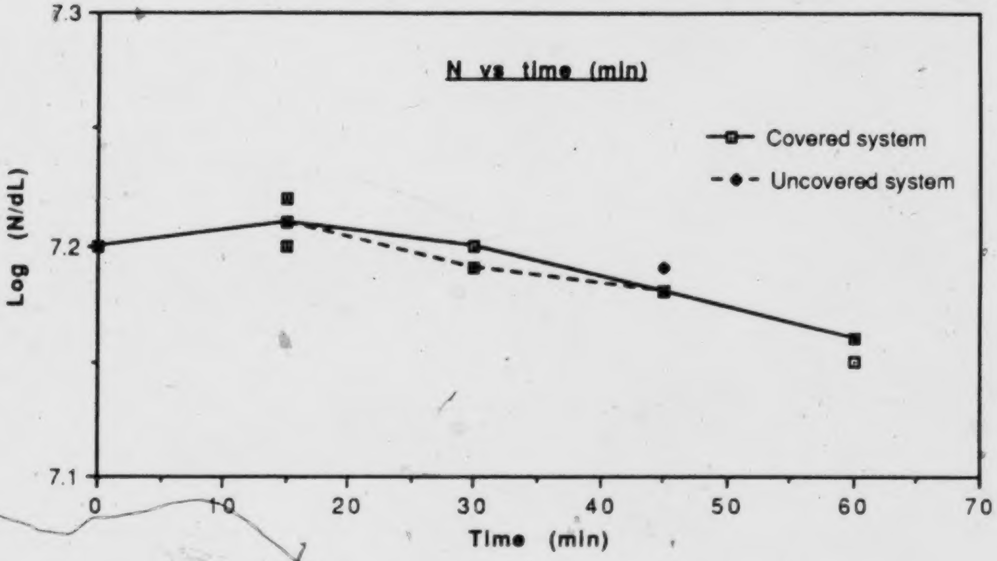


Fig. 4.10

BACTERIAL SURVIVAL IN 0.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^7.2 CFU/dL, pH 6.9)



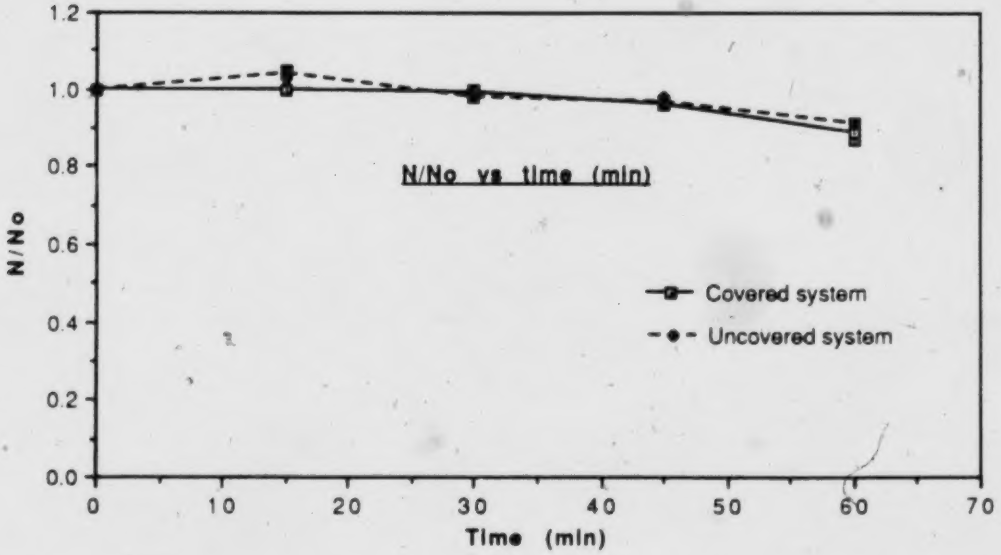
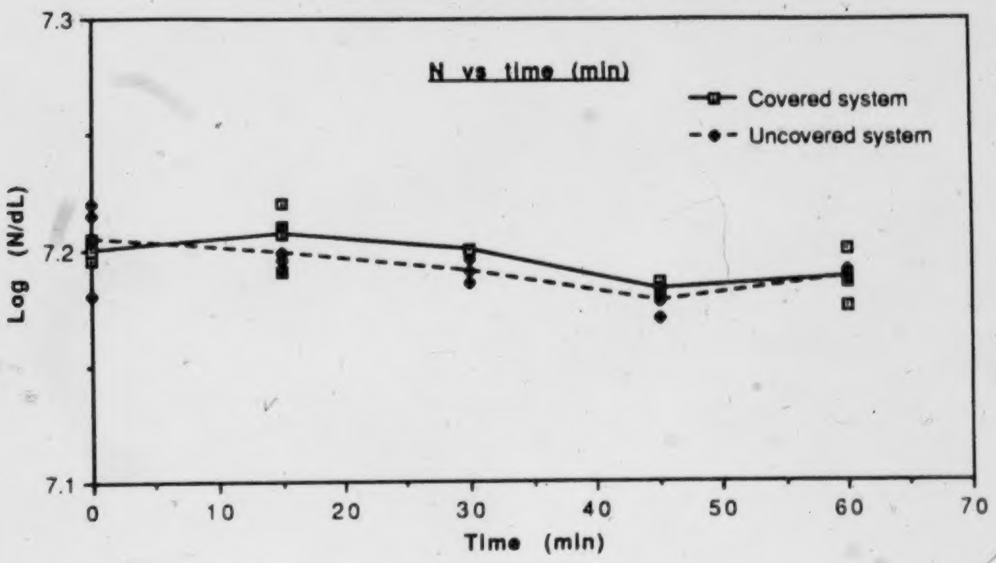
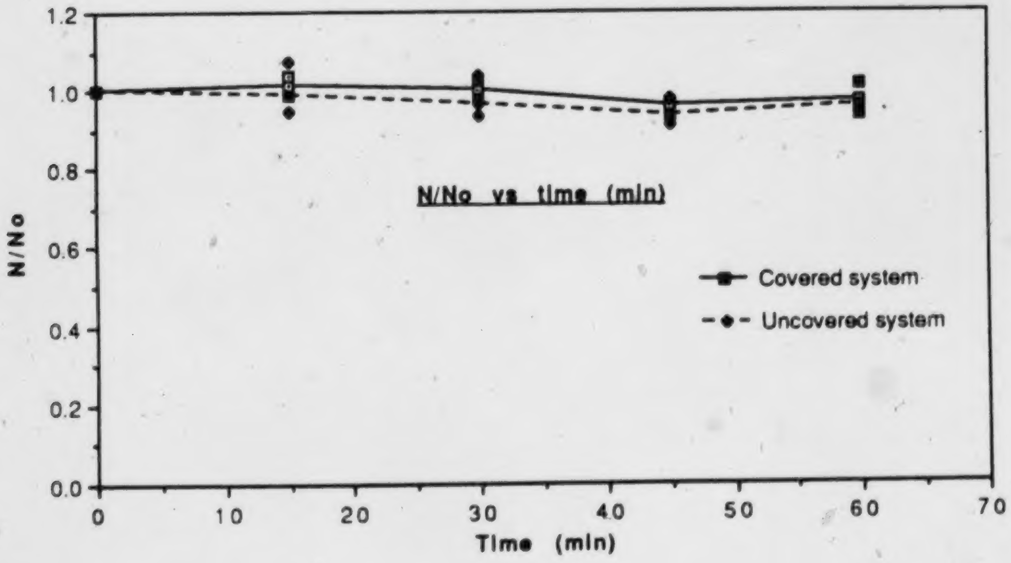


Fig. 4.11

BACTERIAL SURVIVAL IN O.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^7.2 CFU/dL, pH 9)





> "whether or not this is the sole explanation of the lethal effects, the fact remains that most of the cells affected are not killed instantly but rather die after a definite lapse of time".

In literature, not much experimental data is available to support this philosophy expect that Benarde (1965) observed that, in one case, when the pH was increased from 7 to 9; the disinfection efficiency of ClO_2 increased. Since chlorine dioxide neither dissociates nor disproportionate into other chemical species in this range, he, therefore, related this phenomena with the change in sensitivity of the microorganisms. Similarly, Morris (1970) reported that Wuhrman and Zobrist observed 4 to 5 times more bacterial kill with silver ions when the pH of the solution was increased from 6.3 to 8.7. Since there were no known or likely effects of pH on Ag^+ in this range, he postulated that probably bacteria were the source of this effectiveness.

It was, however, found from this study that the variations in pH value from 4 to 9 of the 0.05 M phosphate buffer solution did not affect the survival of *E. coli* significantly, at 5% level, up to 60 min.

4.3 EFFECTS OF pH ON OZONE DISINFECTION:

The effects of pH variations on the *E. coli* survival at a uniform ozone dose were observed in 56 trials. An average of 46 µg/L of ozone dose was applied to an average initial microbial density of $10^{7.2}$ CFU/dL. The pH levels of the 0.05 M phosphate buffer solutions were 4, 6.9 and 9. Samples were drawn at times 30, 60 and 120 sec to determine the effects of applied dose on bacterial survival. The data is given in Table 4.6; and raw data is presented in appendix 4. The observations from this series of experiments were plotted in terms of ozone consumed vs. time (Figures 4.12 to 4.14) and bacterial survival vs. time and ozone used during the disinfection process (Figures 4.15 to 4.17).

It was observed that the total ozone consumed was slightly higher at pH 6.9 than at pH 4 at all times and similarly the total ozone consumption was higher at pH 9 than at pH 4 and 6.9. This difference is quite visible in the graphs but is not significant at 5% significance level. The results also revealed that the rate of ozone disappearance from the disinfection system was proportional to the pH of the system. The ozone consumption in actual killing of bacteria was also calculated using the following relationship:

O_3 dose applied = O_3 used in disinfection + residual O_3
concentration + O_3 decomposed + O_3 stripped
off to the atmosphere

It was found that the ozone used in disinfection was always less than the total ozone utilized in the process indicating that some of the ozone was lost via non-disinfection processes. Further, the rate of ozone consumption in open vessels was higher than in the covered vessel.

The disinfection curves gave essentially the same shape at all three different pH values indicating two distinct stages of dose-response relationships. In the beginning, the bacterial kill was very

TABLE : 4.6

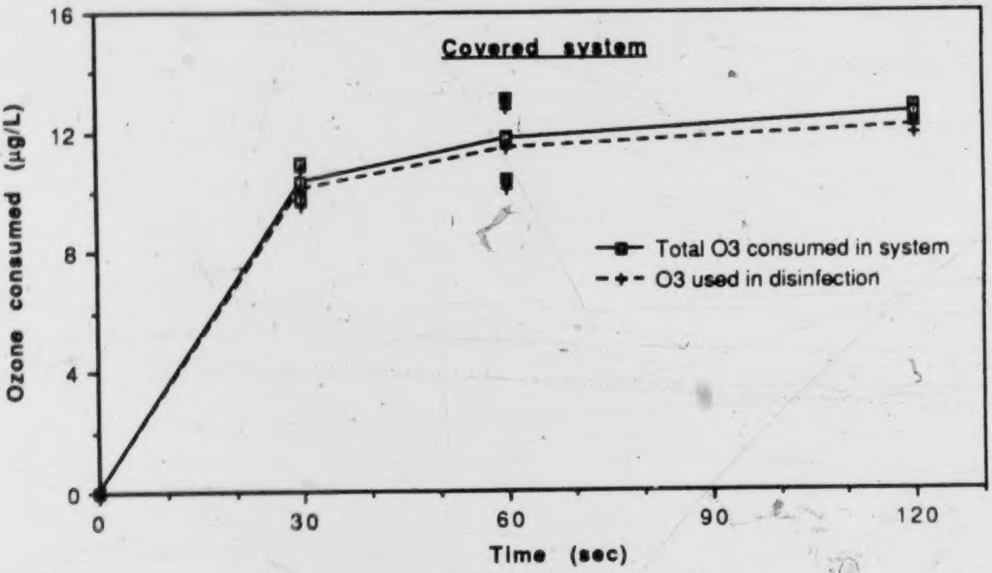
EFFECTS OF pH VARIATIONS ON OZONE DISINFECTION

pH	Log No/dL	System	Contact time (sec)	Number of trials	Mean ozone used in disin-fection (ug/L)	Mean ozone* decomposed (ug/L)	Mean OH radicals produced (log units) 1:1 ratio	1:0.5 ratio	Mean E. coli survival (log N/No)	95% confidence limits for Log N/No Lower	Higher
4.0	7.2	C	30	3	10	0.21	13.42	13.12	-3.70	-3.73	-3.67
		U		3	11	0.24	13.47	13.17	-3.55	-3.66	-3.43
		C	60	3	11	0.32	13.60	13.30	-3.96	-4.12	-3.80
		U		3	12	0.38	13.67	13.37	-3.86	-3.96	-3.77
		C	120	3	12	0.53	13.82	13.52	-5.25	-5.31	-5.20
		U		3	12	0.64	13.90	13.60	-5.18	-5.27	-5.09
6.9	7.2	C	30	4	10	0.15	13.26	12.96	-3.53	-3.57	-3.50
		U		4	10	0.28	13.54	13.24	-3.48	-3.58	-3.37
		C	60	3	11	0.30	13.57	13.27	-3.87	-4.05	-3.69
		U		3	11	0.48	13.78	13.48	-3.86	-4.16	-3.56
		C	120	3	12	0.61	13.88	13.58	-5.17	-	-
		U		3	12	0.95	14.07	13.77	-5.14	-5.19	-5.09
9.0	7.2	C	30	3	10	0.27	13.52	13.22	-3.37	-3.48	-3.25
		U		3	10	0.48	13.78	13.48	-3.28	-3.36	-3.20
		C	60	3	12	0.69	13.94	13.64	-3.54	-3.66	-3.41
		U		3	12	0.91	14.06	13.76	-3.47	-3.63	-3.31
		C	120	3	15	1.39	14.24	13.94	-4.91	-4.95	-4.87
		U		3	14	2.05	14.41	14.11	-4.83	-4.88	-4.77

* See appendix 5   C for covered system   U for uncovered system

Fig. 4.12

OZONE CONSUMPTION IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING E. coli (No ~ 10^7.2 CFU/dL, Co ~ 46 μg/L, pH 4)



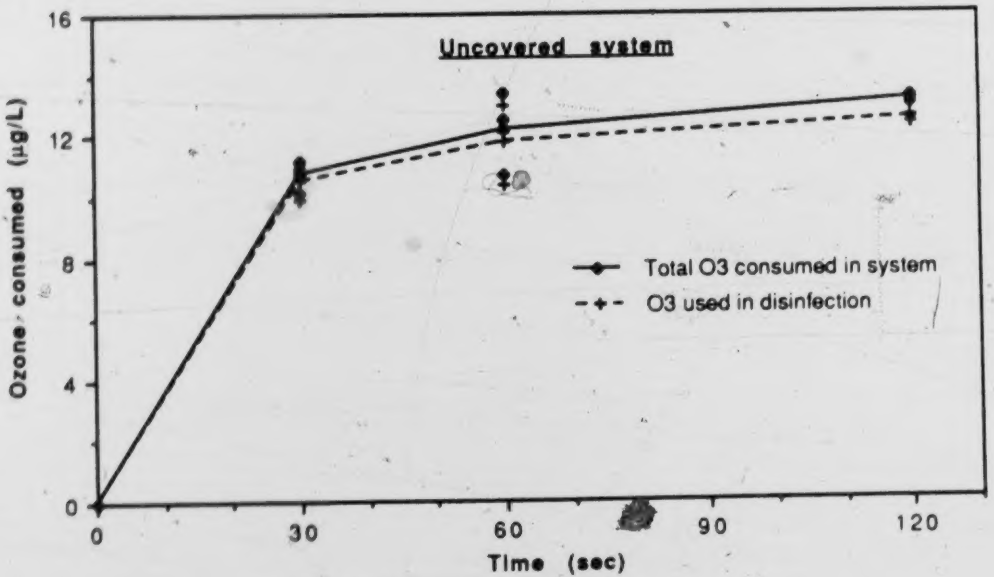


Fig. 4.13

OZONE CONSUMPTION IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING E. coli (No~10^7.2 CFU/dL, Co~46 µg/L, pH 6.9)

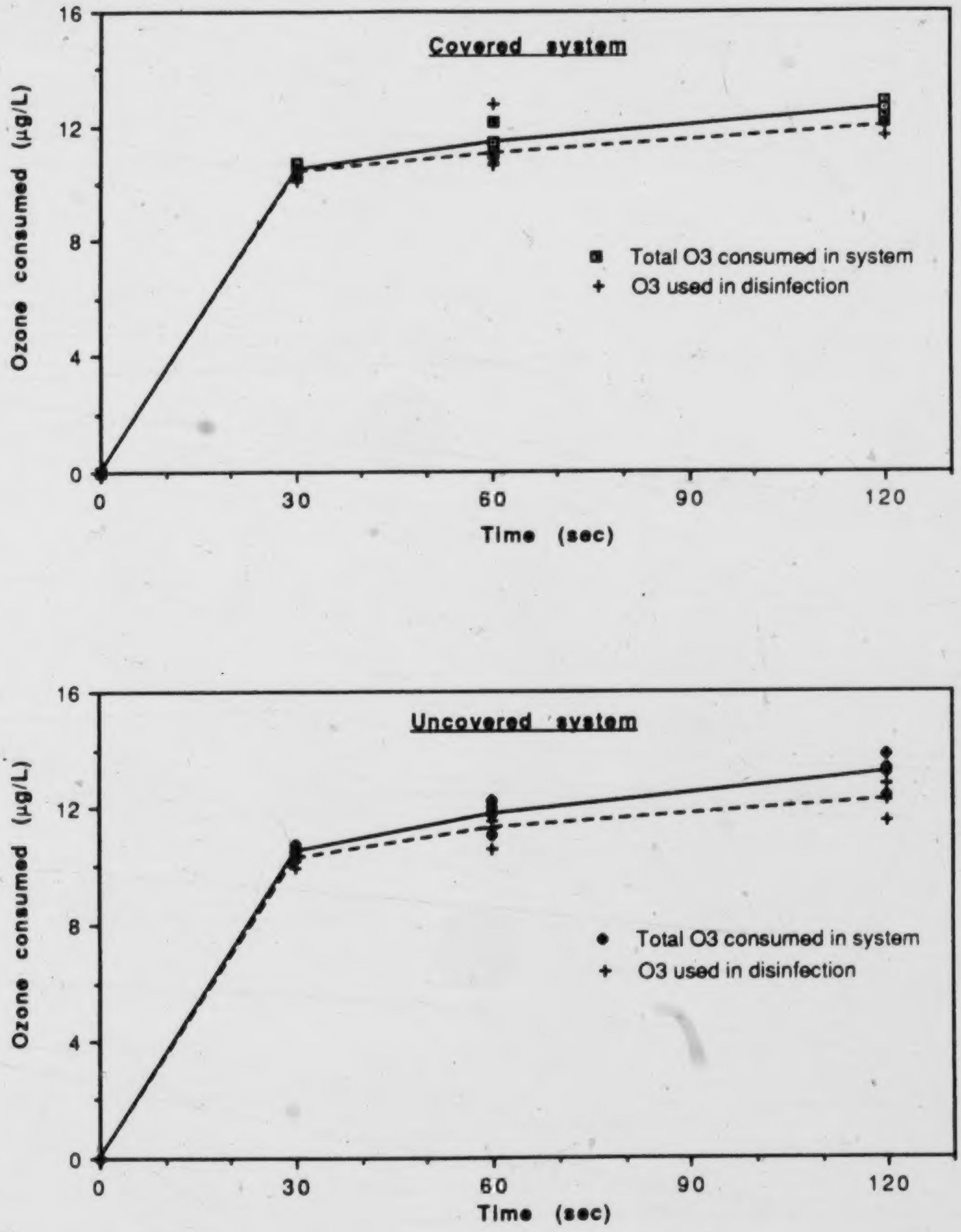
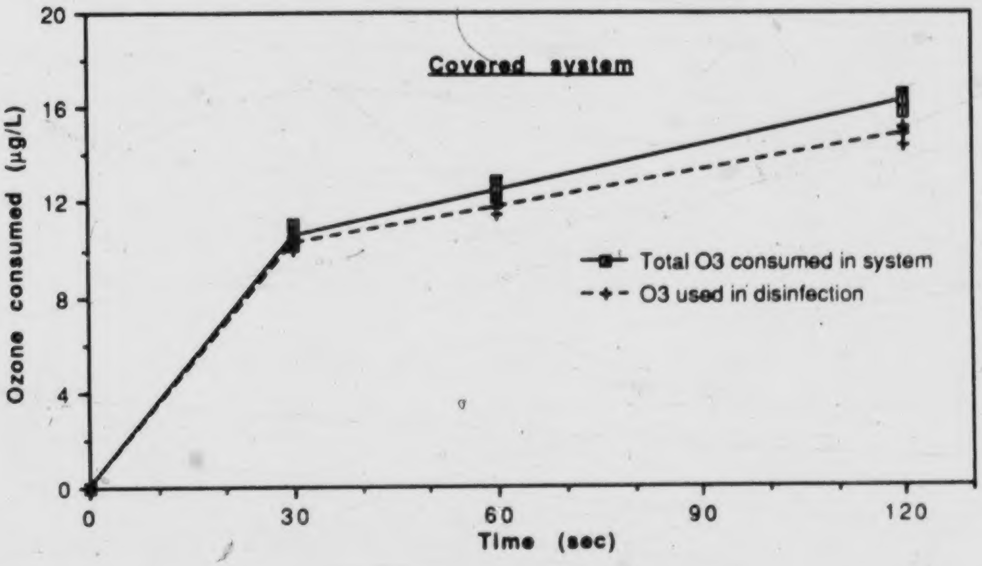


Fig. 4.14

OZONE CONSUMPTION IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING E. coli (No ~ 10^7.2 CFU/dL, Co ~ 46 µg/L, pH 9)



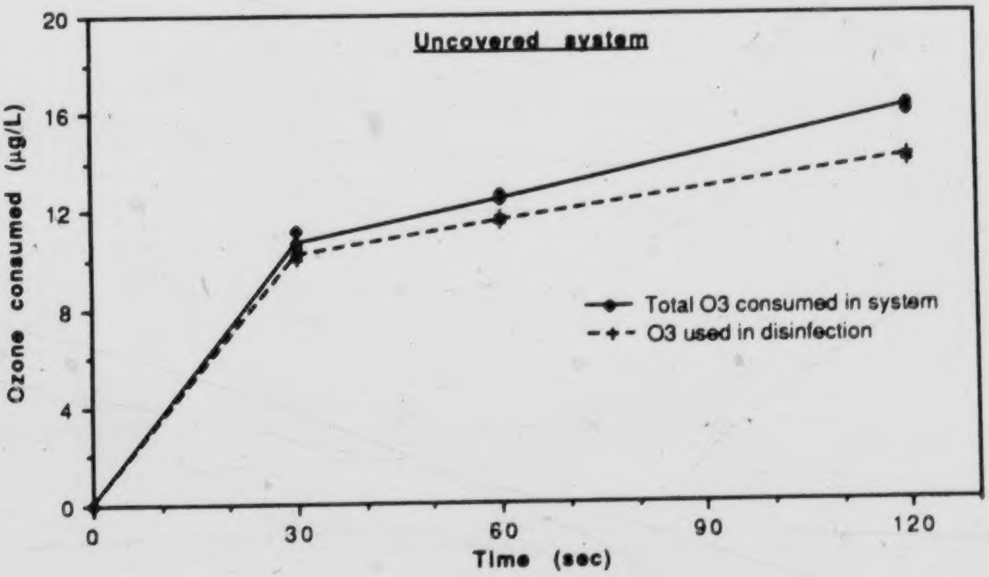


Fig. 4.15

DOSE-RESPONSE IN 0.05 M PHOSPHATE BUFFER SOLUTION
(No ~ 10^7.2 CFU/dL, Co ~ 46 µg/L, pH 4)

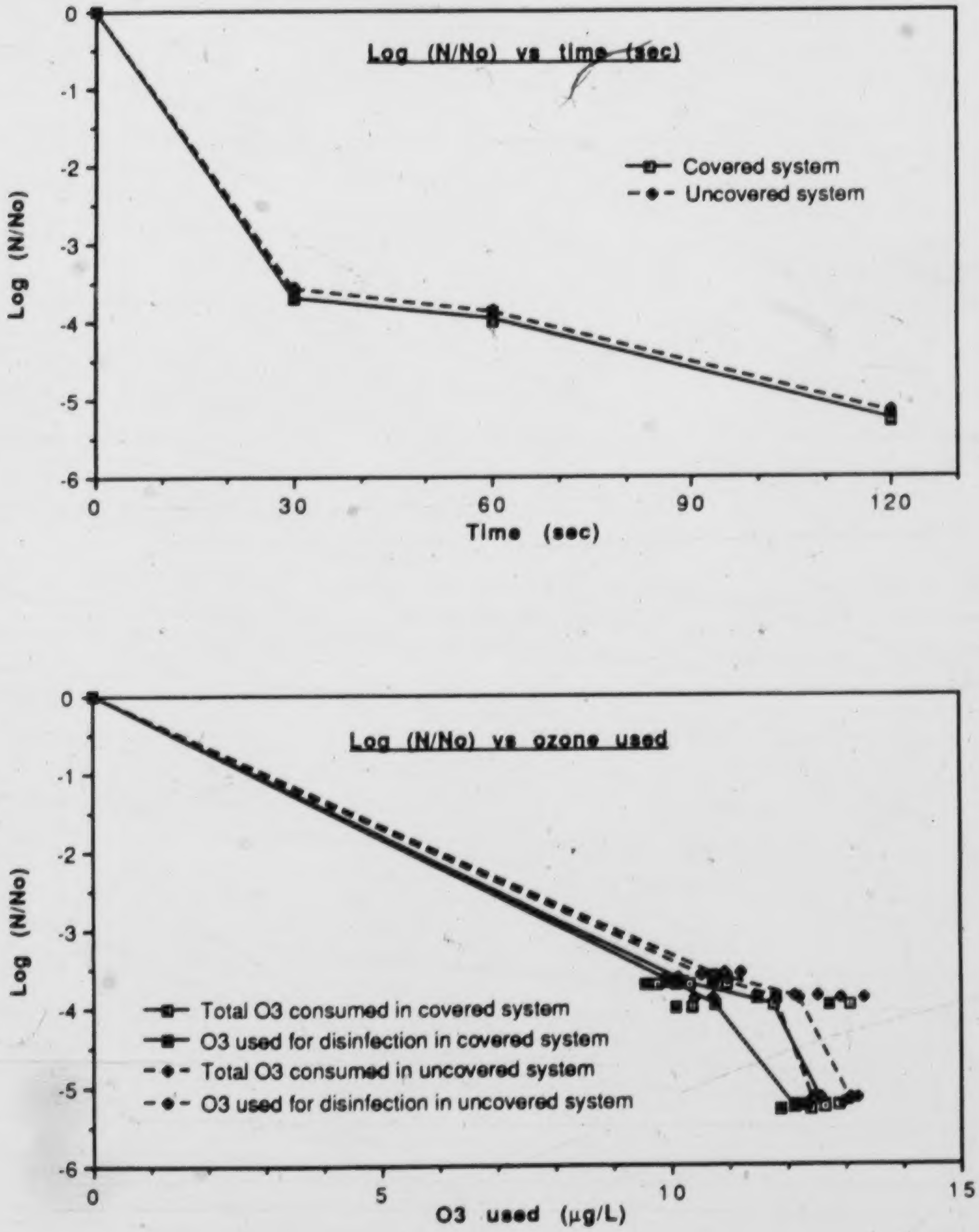
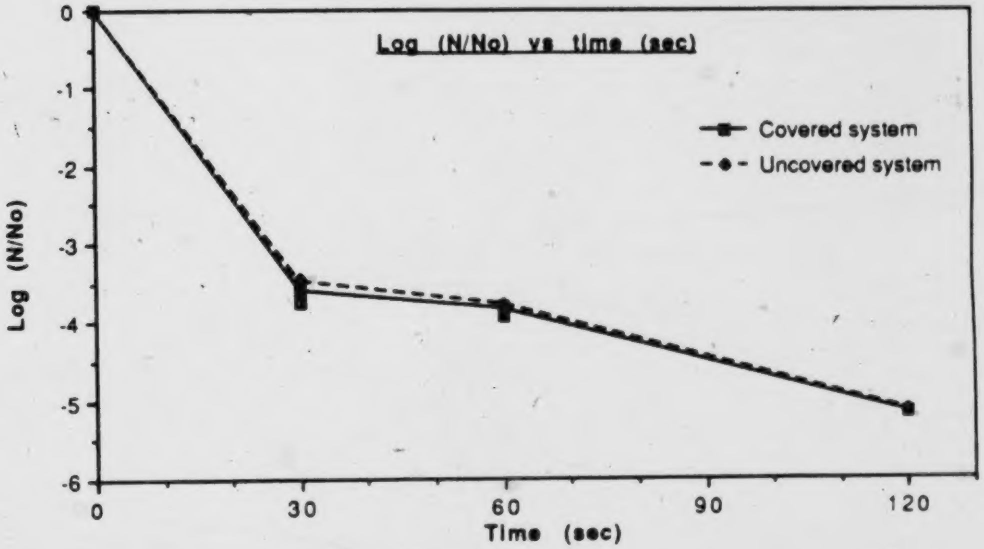


Fig. 4.16

DOSE-RESPONSE IN 0.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^7.2 CFU/dL, Co ~ 46 μg/L, pH 6.9)



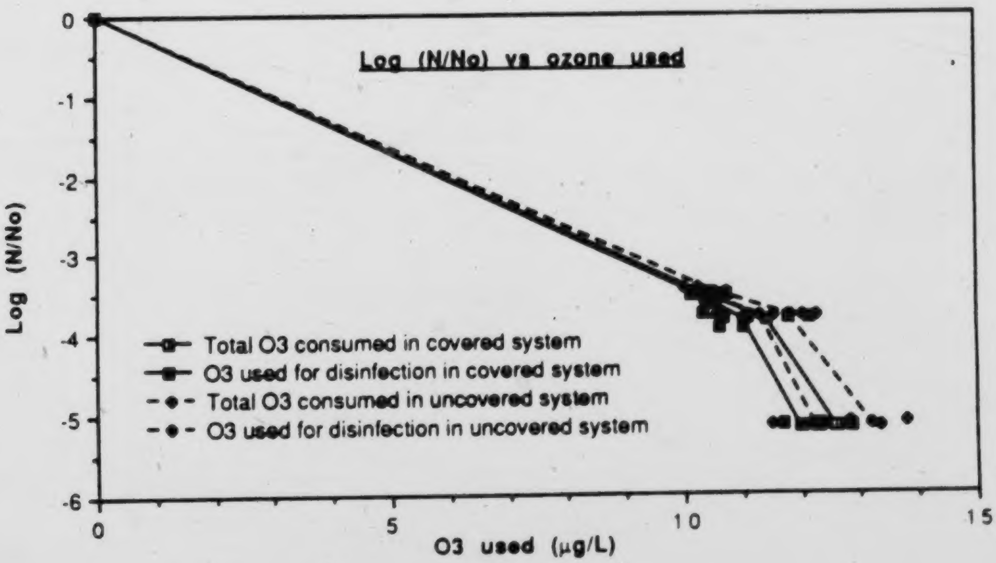
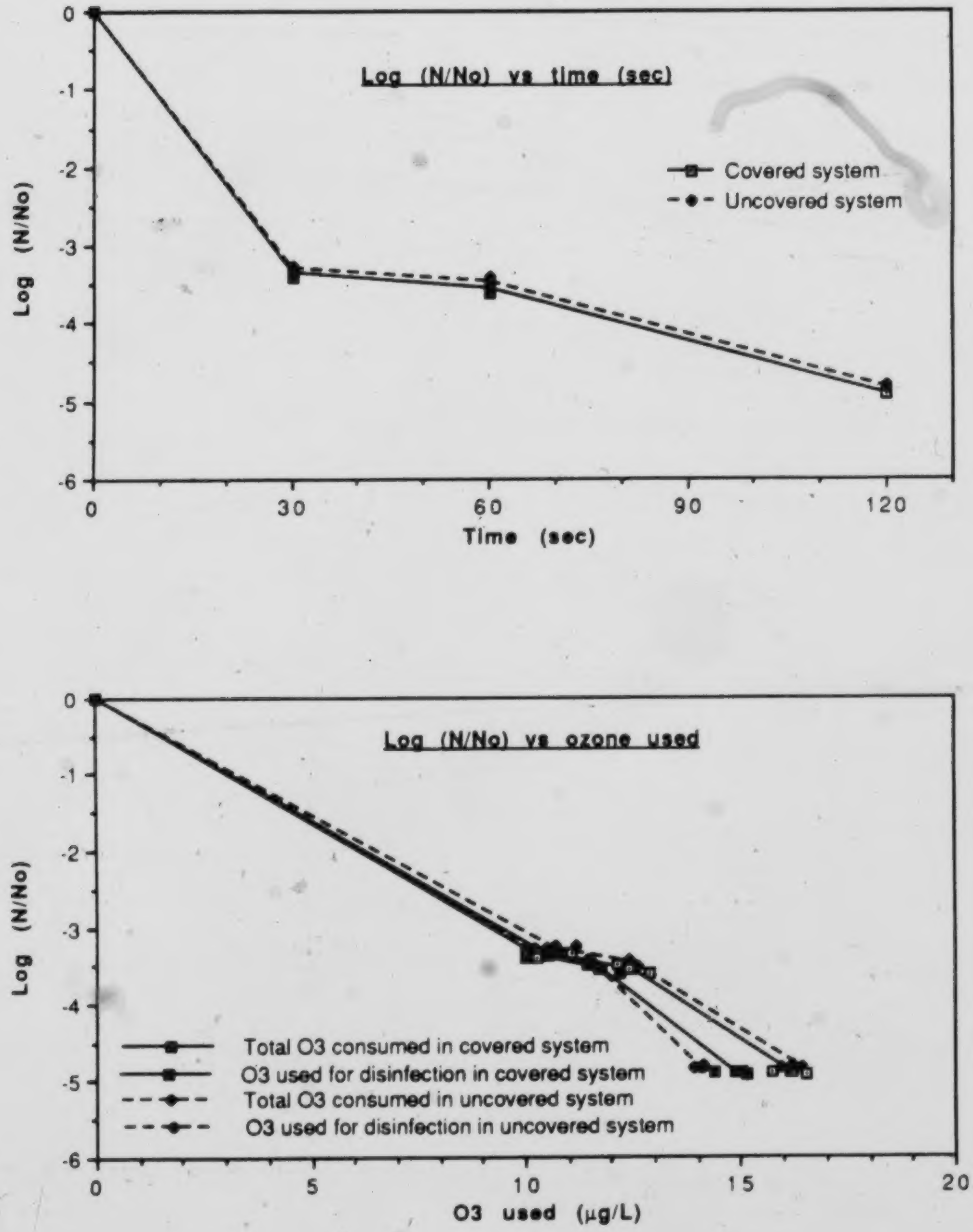


Fig. 4.17

DOSE-RESPONSE IN 0.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^7.2 CFU/dL, Co ~ 46 μg/L, pH 9)

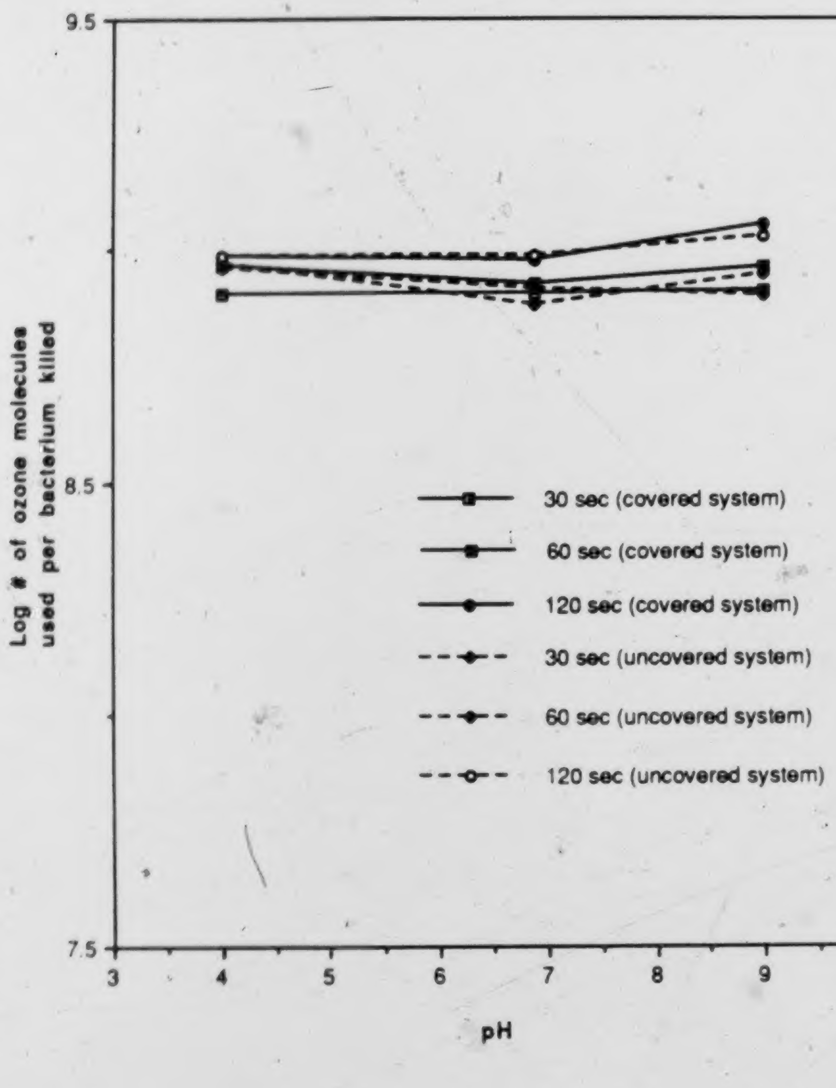


fast as indicated by Hoigne (1982). After first stage of reaction, when enough cytoplasmic material was released from the lysed cells and was available to react with the ozone molecules, a competition existed between lysed material and bacterial cells for ozone. Ozone consumption continued during this stage but the rate of bacterial kill gradually decreased until the disinfection process was terminated. The amount of ozone consumed was calculated in terms of number of ozone molecules used to kill one bacterium and virtually the same number (8×10^8) were found to be utilized to remove one bacterium at all pH levels used (Figure 4.18). It was also found that the variations the in pH of the solution varied the total ozone consumption in the system but it did not have any effect on the survival of *E. coli* and the ozone molecules required to kill one bacterium were same at different pH values, provided the other conditions were kept unchanged. These findings were in agreement with the available literature.

Rose (1965) mentioned that the presence of H^+ ions is an essential requirement of the microorganisms in order to grow, but these requirements are quite low, and in higher concentrations the ions may have a toxic or lethal effect on the microorganisms. Bacteria, with some expectations, prefer media of pH values near neutrality and can not usually tolerate pH values much below 4 to 5. It is well documented in the literature (Rose, 1965) that the cytoplasmic membrane in microorganisms is relatively impermeable to H^+ and OH^- ions; thereby, the concentration of these ions probably remains reasonably constant in the cytoplasm even when the pH of

Fig. 4.18

NUMBER OF OZONE MOLECULES USED PER BACTERIUM KILLED FOR DIFFERENT pH VALUES (No ~ log 7.2 CFU/dL)



the surrounding medium is varied quite widely. Rose (1965) further mentioned that the pH value of the medium probably affects microbial activity due to the interaction between H^+ ions and enzymes and enzyme carrier molecules in the cytoplasmic membrane.

Smith and Bodkin (1944) found no difference in the bacterial kill, using ozone, due to the pH changes from 5 to 9. Diaper (1972) has reported that the pH variations of the disinfection medium did not affect the bactericidal potential of ozone, while in chlorination, this was considerably affected due to these variations. Farooq *et al.* (1977) studied the effects of pH and temperature on the ozone disinfection. They concluded that if the ozone residual remains constant, the disinfection capability would not be affected by the change in pH.

It can be summarized from these studies that since many disinfectants, like HOCl, are more potent in their undissociated form, which itself is a function of pH, so it can be said that pH affect the disinfection potential of those via dissociating them. Ozone does not dissociate with the variations in pH, so its disinfection potential does not change with the changes in the pH of the system. However, there is a similarity between chlorination and ozone disinfection that at low pH values, less amounts of both chlorine and ozone are required to achieve a desired kill. This theory is in agreement with the results obtained from the present study which demonstrates that the total amount of ozone used in disinfection is a function of system conditions. But because the ozone autodecay is a direct function of pH, so the increase in the pH value of the disinfection system results in less molecular ozone available for killing the bacteria and, consequently, the disinfection becomes a low yield process. As far as the amount of ozone is used, calculated in terms of ozone molecules, it remained the same to kill one bacterium regardless of the pH value of the system.

4.4 EFFECTS OF OH° RADICALS ON OZONE DISINFECTION:

Because there is a controversy over the role of OH° radicals in ozone disinfection, an attempt was made to relate the amount of hydroxyl radicals produced in the system, as a result of ozone decomposition, with the bacterial kill. As the ozone decomposition is a function of pH, so higher is the pH, higher will be ozone decomposition rate and consequently, higher is the amount of OH° radicals in the system. It is known that one decomposed ozone molecule gives 0.5 molecules of OH° radicals (Hoigne, 1975), therefore, the amount of OH° radicals was calculated from the decomposed ozone (Table, 4.7; appendix, 5). The relationships between total OH° radicals in the system and bacterial kill were plotted, in Figure 4.19, for covered and uncovered systems.

Apparently, these graphs show that the bacterial survival is a function of the amount of the OH° radicals up to a certain stage and once the threshold level is met, their action becomes independent of their concentration. The same pattern was observed at all three levels of pH. This action may be called similar, up to a certain

Table: 4.7

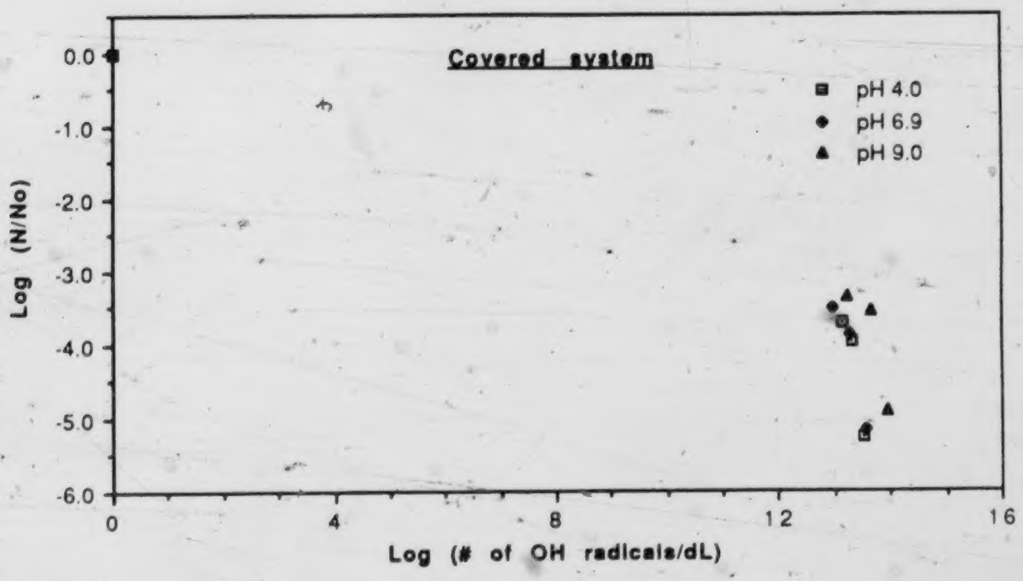
EFFECTS OF pH VARIATIONS ON OH° RADICALS PRODUCTION IN DISINFECTION PROCESS

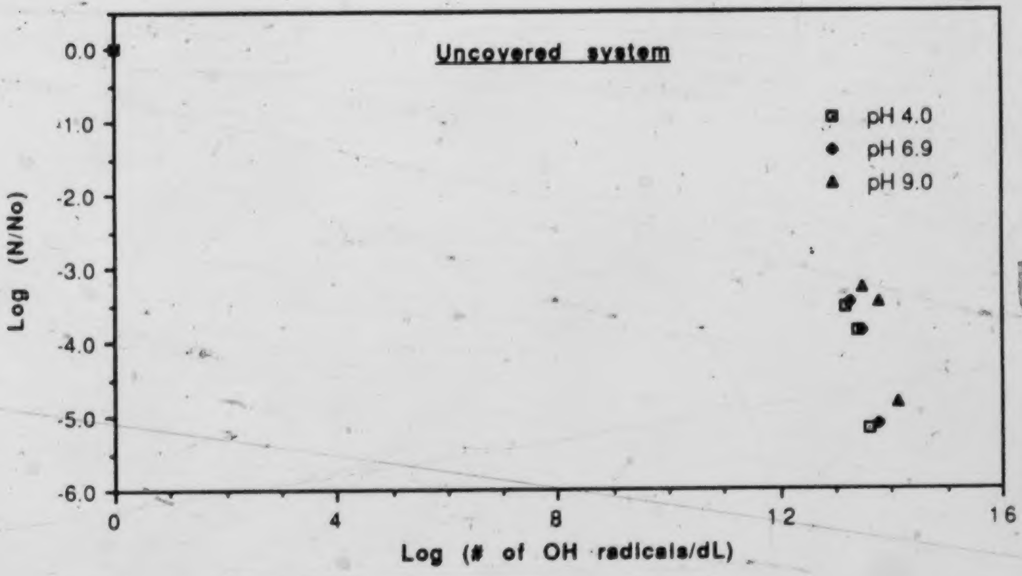
pH	Log No/dL	Contact time (sec)	System	Number of trials	Mean Bacterial survival (log N/No)	Mean ozone* decomposed (ug/L)	Mean OH radicals produced (log units) 1:1 ratio	1:0.5 ratio
4.0	7.2	30	C	3	-3.70	0.21	13.42	13.12
			U	3	-3.55	0.24	13.47	13.17
		60	C	3	-3.96	0.32	13.60	13.30
			U	3	-3.86	0.38	13.67	13.37
		120	C	3	-5.25	0.53	13.82	13.52
			U	3	-5.18	0.64	13.90	13.60
6.9	7.2	30	C	4	-3.53	0.15	13.26	12.96
			U	4	-3.48	0.28	13.54	13.24
		60	C	3	-3.87	0.30	13.57	13.27
			U	3	-3.86	0.48	13.78	13.48
		120	C	3	-5.17	0.61	13.88	13.58
			U	3	-5.14	0.95	14.07	13.77
9.0	7.2	30	C	3	-3.37	0.27	13.52	13.22
			U	3	-3.28	0.48	13.78	13.48
		60	C	3	-3.54	0.69	13.94	13.64
			U	3	-3.47	0.91	14.06	13.76
		120	C	3	-4.91	1.39	14.24	13.94
			U	3	-4.83	2.05	14.41	14.11

* See appendix 5 C for covered system U for uncovered system

Fig. 4.19

EFFECTS OF TIME AND pH VARIATIONS ON BACTERIAL SURVIVAL (in terms of OH radicals production)
O3 decomposition: OH radicals production = 1:0.5
(No ~ 10^7.2 CFU/dL, Co ~ 46 µg/L)





degree, with the all-or-none mechanism of ozone disinfection as proposed by Fetner and Ingols (1956). However, theoretically, as the increase in pH generated more OH^o radicals, more kill was expected, but, graphs reflect that there was no significant difference in the bacterial survival. This gives a thought that probably there was no relationship between the amount of OH^o radicals and the bacterial kill and the observation mentioned was just a coincidence. Thus no definite result can be drawn regarding the disinfection potential of OH^o and, in fact, to establish their role in disinfection process, a different kind of experimental set up is required where OH^o radicals are used as a sole source of bactericidal agent.

From literature, it appears that there is no direct relationship between the germicidal activity of the substance and its oxidation potential. For example, there are lot of antimicrobial agents which kill the microorganisms but are not oxidants at all. On the other hand, hydrogen peroxide, MnO_4^- and Mn^{+++} are good oxidants but do not carry good disinfection potential. The germicidal activity of molecular HOCl does not change over the pH range of 3 to 9 even though its oxidation potential changes by more than 0.3 volts (Morris, 1970). Molecular iodine, I_2, has the same germicidal potential as HOCl, but the redox potential of two is quite different. I_3^-, having the same oxidation potential as I_2, posses much less disinfection potential. Similarly, molecular oxygen, O_2 has a strong oxidation potential of 1.27 volts, which is much greater than many effective germicides, but is not effective as a disinfectant.

In spite of the above facts, attempts have been made to correlate the germicidal activities of the oxidant with their oxidation potential either in terms of standard redox potential of the germicidal substance or some measured EMF in the working solution (Morris, 1970). Each had popularity for some time, but no definite conclusion could be drawn, because the germicidal activity is predominantly kinetic in nature, once the thermodynamic requirement of the process has been met; while the redox potential is simply another expression for a change in free energy. Hence, the relationships between the germicidal activity and the oxidation potential of a substance may be coincidental in nature for some system and lead to an error or confusion if an attempt is made to extrapolate them for other studies.

4.5 EFFECTS OF INITIAL BACTERIAL DENSITIES ON BACTERIAL DIE-OFF:

To observe the effects of variations in bacterial densities on their die-off, studies were conducted using 0.05 M phosphate buffer solution at pH 6.9 and with initial bacterial densities of $10^{4.2}$ CFU/dL, $10^{7.2}$ CFU/dL and $10^{9.3}$ CFU/dL. The die-off studies were also conducted with the addition of sodium thiosulphate in the phosphate buffer solution, using an initial density of $10^{7.2}$ CFU/dL.

The summary of die-off data is given in Table 4.8, and graphs are shown in Figures 4.20 to 4.23. The raw data is presented in Appendix 6. The graphs revealed that there were small variations in

Table: 4.8 (A)

EFFECTS OF BACTERIAL DENSITIES ON DIE-OFF
(COVERED SYSTEM)

No.	Time (min)	No ~ 10^4.2 (CFU/dL)	No ~ 10^7.2 (CFU/dL)	No ~ 10^9.3 (CFU/dL)
1	0	4.21 - 4.23	7.20	9.27 - 9.34
2	15	4.17 - 4.23	7.19 - 7.23	9.28 - 9.36
3	30	4.20 - 4.21	7.18 - 7.20	9.27 - 9.36
4	45	4.20 - 4.23	7.17 - 7.19	9.26 - 9.32
5	60	4.18 - 4.20	7.13 - 7.16	9.23 - 9.32

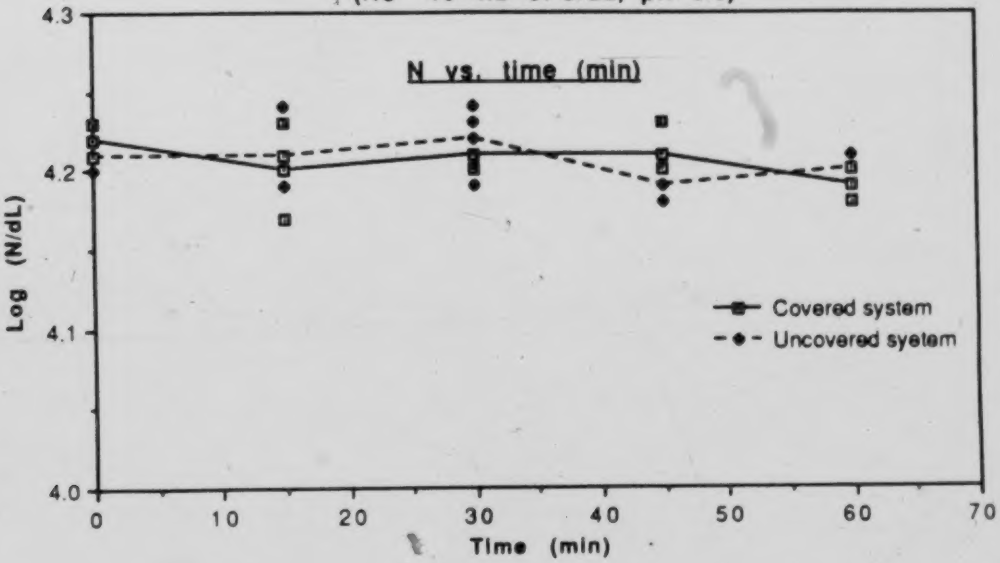
Table: 4.8 (B)

EFFECTS OF BACTERIAL DENSITIES ON DIE-OFF
(UNCOVERED SYSTEM)

No.	Time (min)	No ~ 10^4.2 (CFU/dL)	No ~ 10^7.2 (CFU/dL)	No ~ 10^9.3 (CFU/dL)
1	0	4.20 - 4.23	7.19 - 7.22	9.26 - 9.36
2	15	4.19 - 4.24	7.20 - 7.22	9.27 - 9.32
3	30	4.19 - 4.24	7.18 - 7.20	9.24 - 9.31
4	45	4.18 - 4.20	7.16 - 7.20	9.26 - 9.30
5	60	4.17 - 4.21	7.15 - 7.18	9.23 - 9.29

Fig. 4.20

BACTERIAL SURVIVAL IN
0.05 M PHOSPHATE BUFFER SOLUTION
(No ~10^4.2 CFU/dL, pH 6.9)



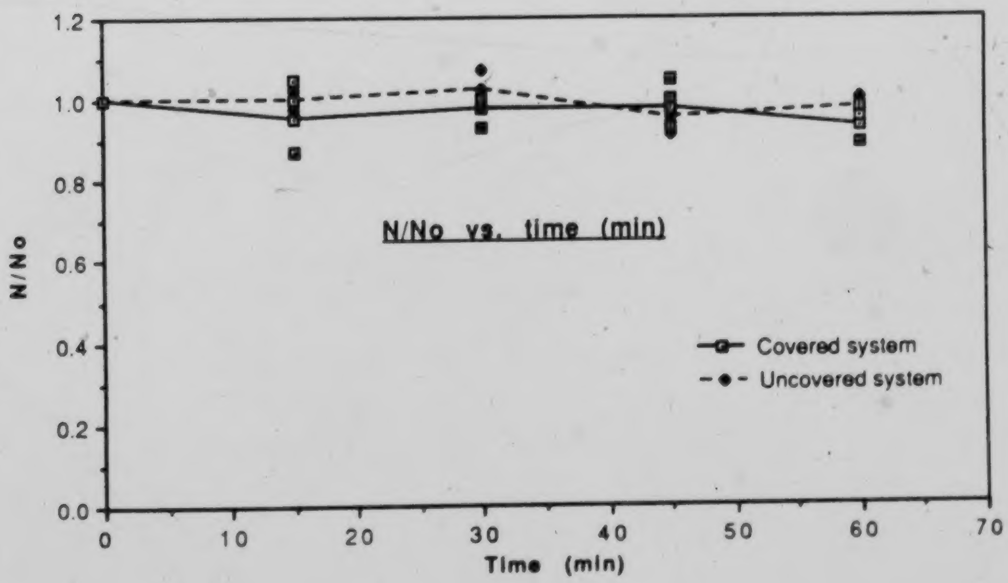
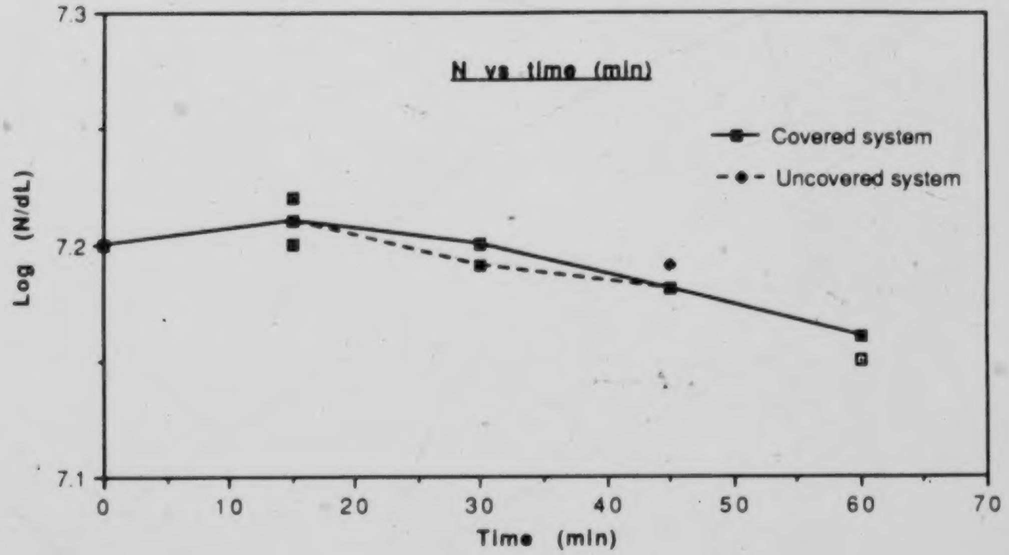


Fig. 4.21

BACTERIAL SURVIVAL IN
0.05 M PHOSPHATE BUFFER SOLUTION
(No ~ 10^7.2 CFU/dL, pH 6.9)



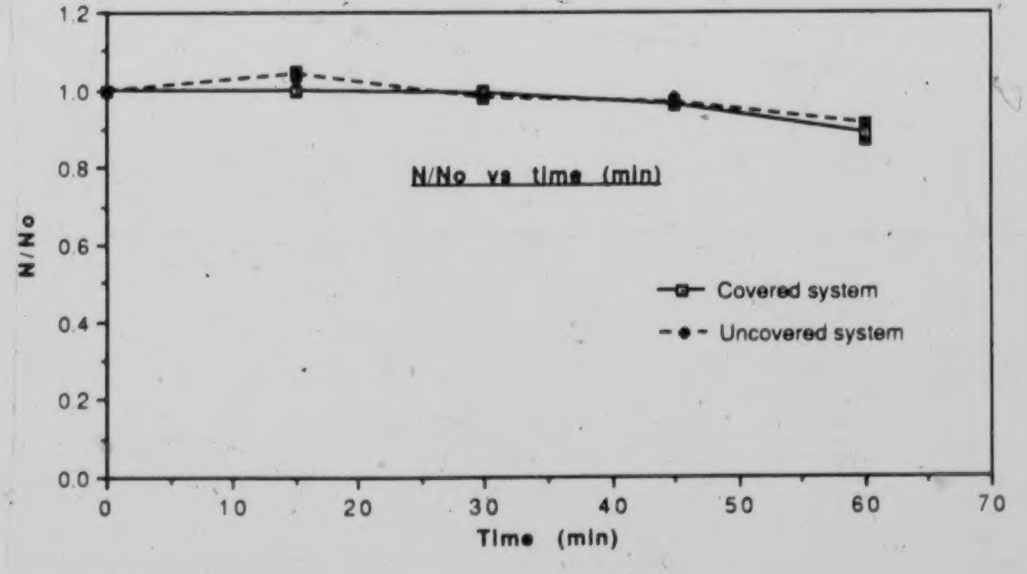
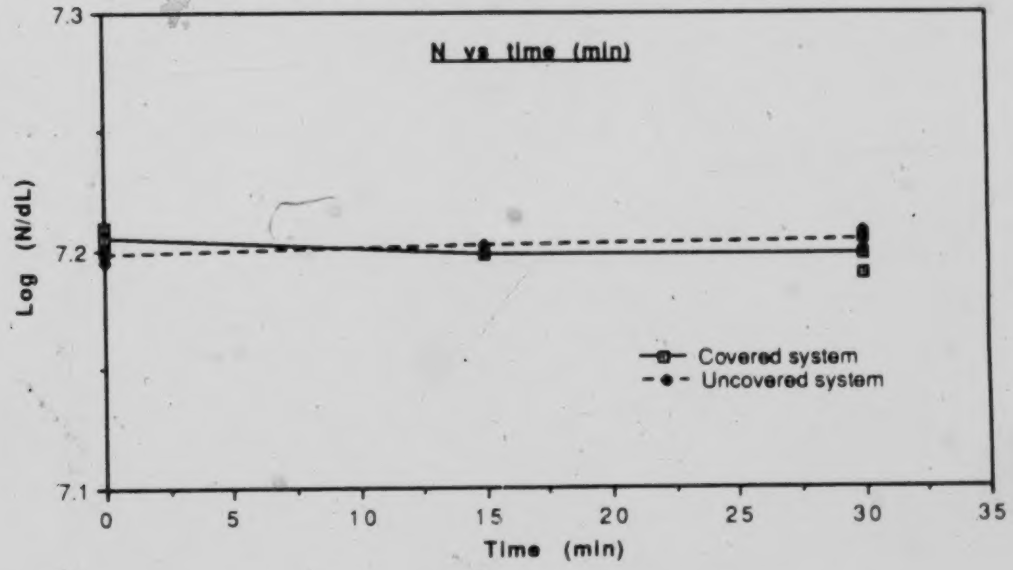


Fig. 4.22

BACTERIAL SURVIVAL IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING SODIUM THIOSULPHATE (No ~ 10^7.2 CFU/dL, pH 6.9)



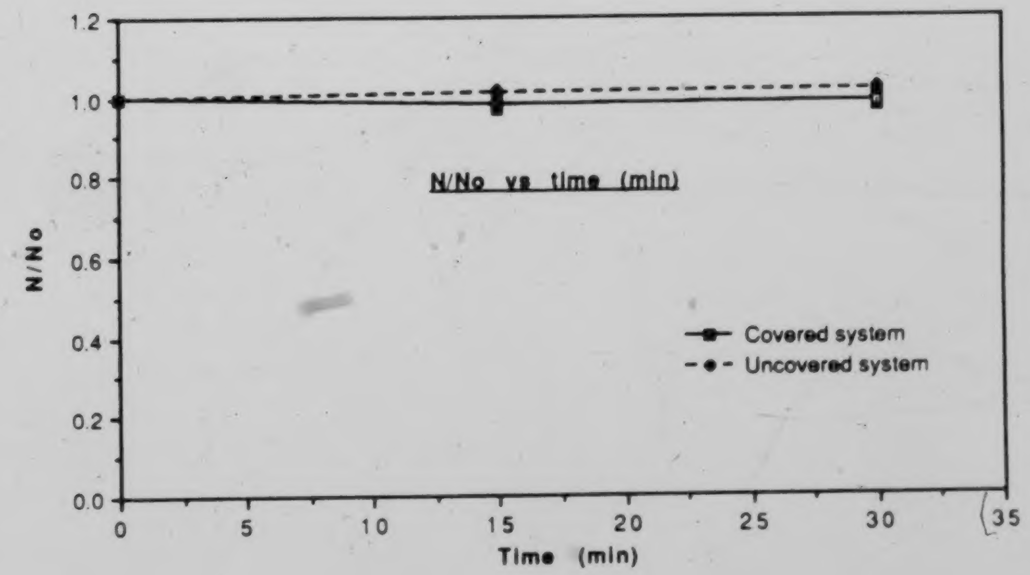
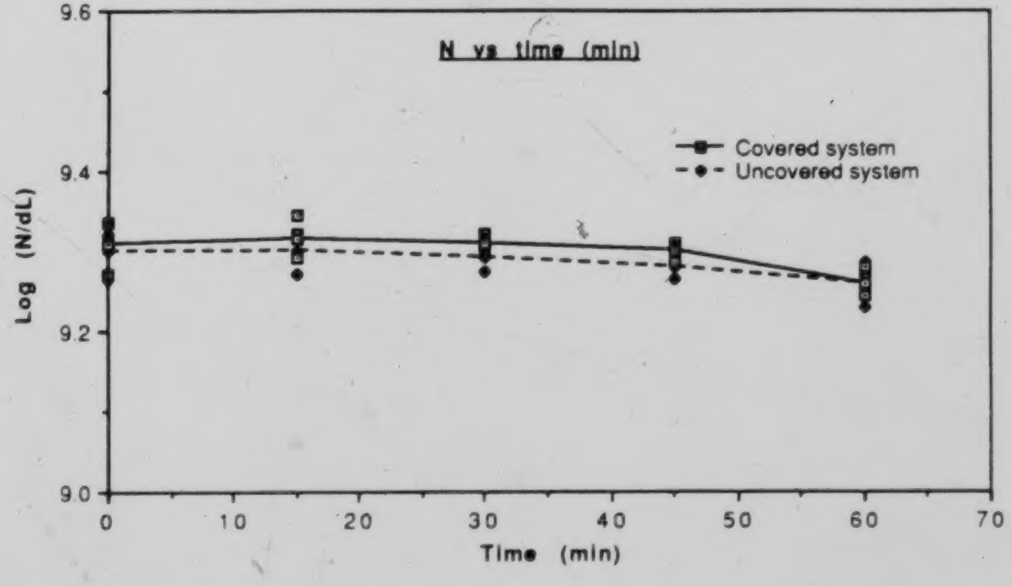
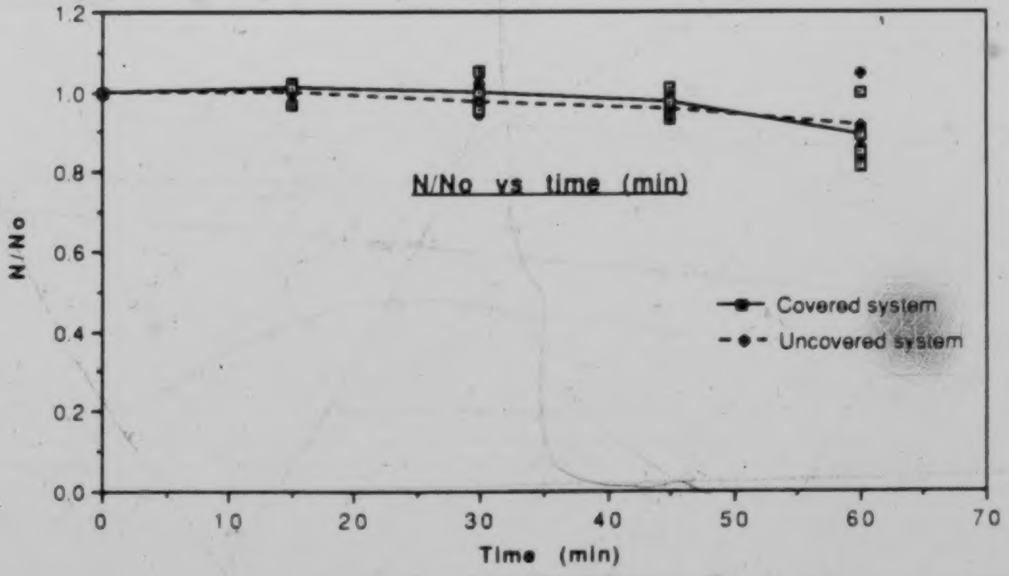


Fig. 4.23

BACTERIAL SURVIVAL IN 0.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^9.3 CFU/dL, pH 6.9)





the number of bacteria suspended in phosphate buffer but the analysis of variance on the data did not show any significance of these variations at 5% level. This indicated that, like pH variations, the variations in the bacterial densities do not exert any adverse effects on their survival. The effects due the addition of sodium thiosulphate in the phospahte buffer solution were also observed on the *E. coli* survival. The bacterial survival found, in this case, was found more stable than in the absence of sodium thiosulphate over 30 min.

4.6 EFFECTS OF INITIAL BACTERIAL DENSITIES ON OZONE DISINFECTION:

The killing process is an interaction between germicidal agent and the organism, so the initial density of microorganism may be of equal importance in determining the rate of ozone disinfection. Many studies are available which indicate the effects of dosages variation on ozone disinfection but it is hard to find more than one study (Farooq *et al.*, 1977) which describes the effects of initial bacterial density on ozone decomposition process. Since they used a yeast culture, *Candida parapsilosis*, and a culture of an acid fast organism, *Mycobacterium fortuitum*, in their studies, so the results of that study are not of much use in ozone disinfection practice where *E. coli* is used as an indicator of bacterial pollution. Keeping in view the limited use of that data it was decided to conduct a series of ozone disinfection experiments in which the effects of constant ozone dose should be monitored on different initial bacterial densities.

The ozone dose used was approximately 46 µg/L in each case and the bacterial densities were $10^{4.2}$ CFU/dL, $10^{7.2}$ CFU/dL, $10^{9.3}$ CFU/dL and 10^{11} CFU/dL. The samples were drawn at 30, 60 and 120 sec from covered and uncovered reaction vessels. The raw data obtained in dose-response experiments is given in Appendix 7, while Table 4.9 summarizes the experimental conditions along with some other information. It was observed (Figures 4.24 to 4.31) that ozone consumptions were more in uncovered systems than covered systems; however, by taking out the ozone disappeared through non-disinfection processes, the ozone utilized in killing the bacteria was almost the same (there was no significant difference for 1% significance level). Over 120 sec total disinfection period, 80 to 96% of the total ozone used in disinfection was consumed in first 30 sec indicating that the initial attack of ozone was very fast and consequently maximum kill was during this phase. After the second stage, the ozone utilization was continued but rate of utilization was much slower than the first stage. At the same time, the rate of bacterial kill was almost negligible after first stage. The different arguments which can be made in this regard could be that:

- not enough bacterial cells were left to react with ozone molecules (e.g. in the case of $10^{4.2}$ CFU/dL),

TABLE : 4.9

EFFECTS OF INITIAL BACTERIAL DENSITIES VARIATIONS ON OZONE DISINFECTION PROCESS

pH	Log No/dL	Contact time (sec)	System	Number of trials	Mean ozone used in disinfection (ug/L	Mean E. coli survival (log N/No)	95% confidence limits for Log N/No Lower	Higher
6.9	4.2	30	C	3	7	-2.96	-3.05	-2.89
			U	3	8	-2.98	-3.05	-2.91
		60	C	3	8	-3.04	-3.09	-2.99
			U	3	8	-3.01	-3.05	-2.98
		120	C	3	8	-3.06	-3.08	-3.04
			U	3	8	-3.09	-3.16	-3.02
6.9	7.2	30	C	4	10	-3.53	-3.57	-3.50
			U	4	10	-3.48	-3.58	-3.37
		60	C	3	11	-3.87	-4.05	-3.69
			U	3	11	-3.86	-4.16	-3.56
		120	C	3	12	-5.17	-	-
			U	3	12	-5.14	-5.19	-5.09
6.9	9.3	30	C	4	17	-0.09	-	-
			U	4	17	-0.09	-0.11	-0.06
		60	C	3	21	-0.09	-0.10	-0.07
			U	3	22	-0.09	-0.12	-0.07
		120	C	3	39	-0.14	-0.15	-0.12
			U	3	38	-0.13	-0.15	-0.12
6.9	11.0	30	C	4	36	-0.01	-0.02	-0.01
			U	3	35	-0.01	-0.02	0.00
		60	C	3	40	-0.02	-0.02	-0.02
			U	4	39	-0.01	-0.02	0.00
		120	C	3	44	-0.02	-0.03	0.00
			U	4	44	-0.01	-0.03	0.00

Fig. 4.24

OZONE CONSUMPTION IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING E. coli (No~10^4.2 CFU/dL, Co~46 μg/L, pH 6.9)

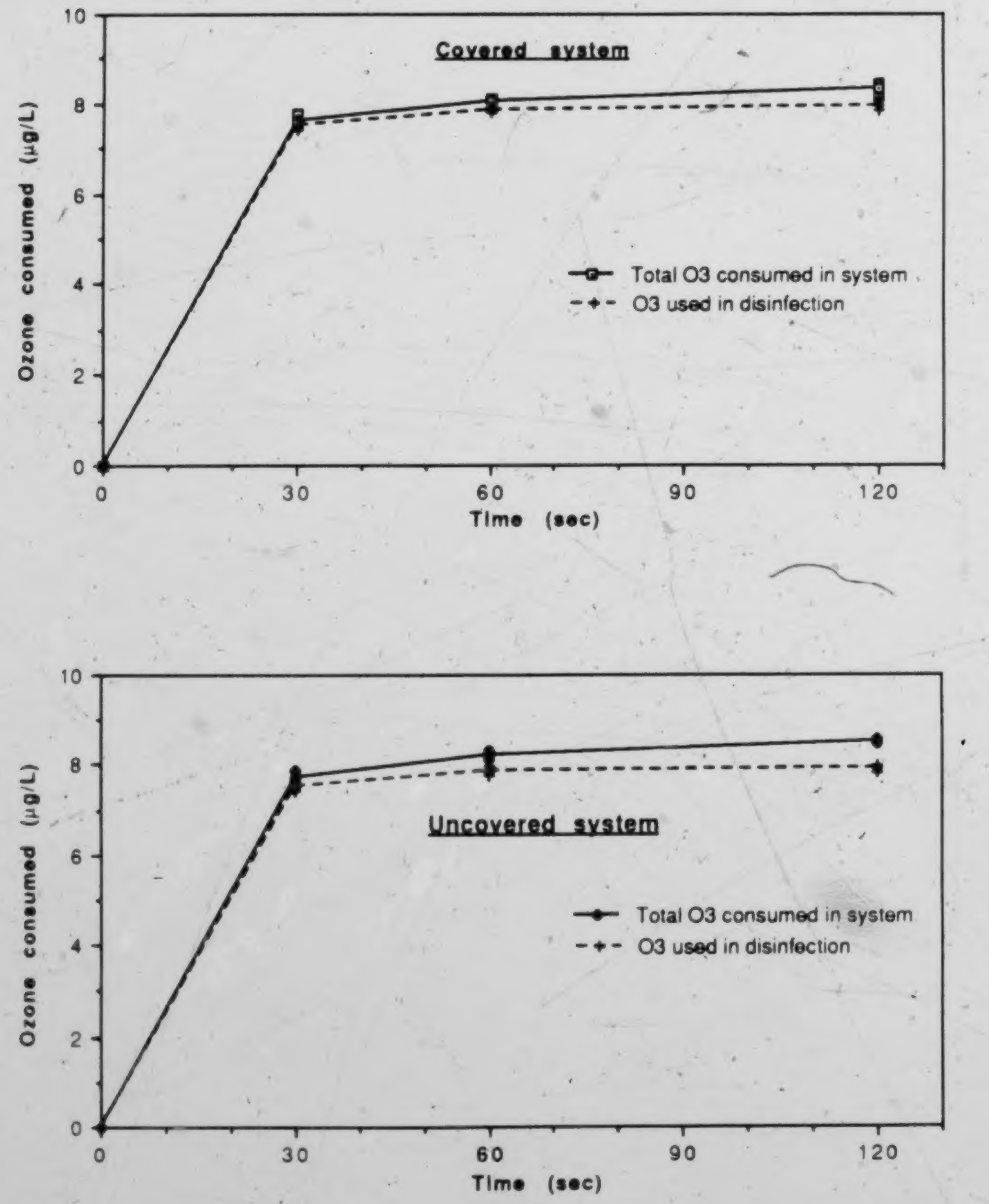
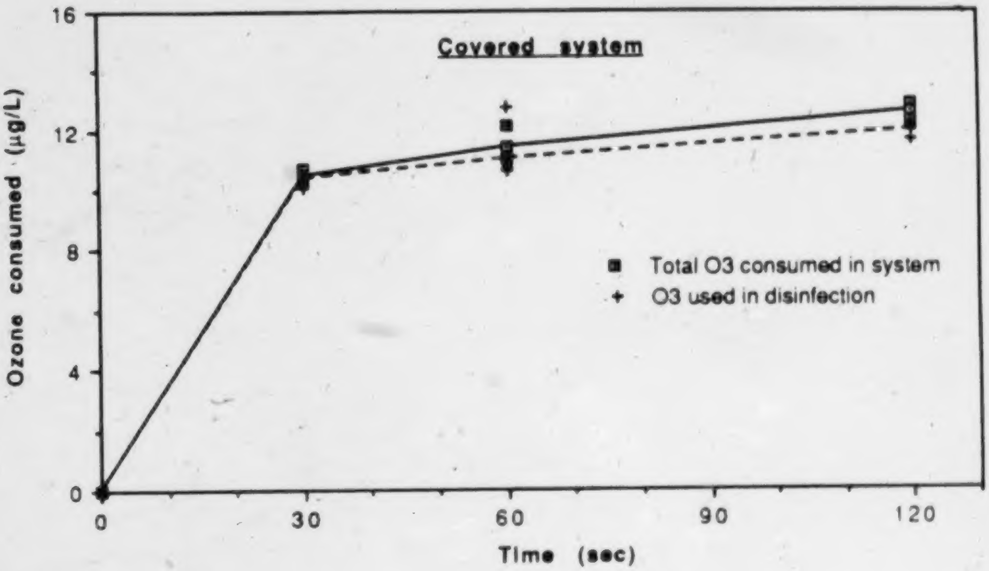


Fig. 4.25

OZONE CONSUMPTION IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING E. coli (No ~10^7.2 CFU/dL, Co~46 µg/L, pH 6.9)



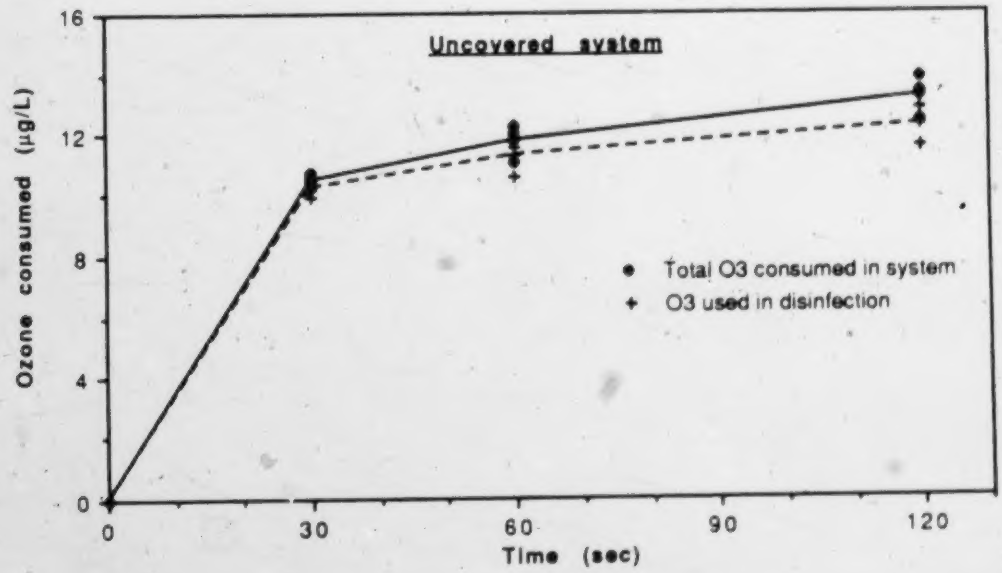
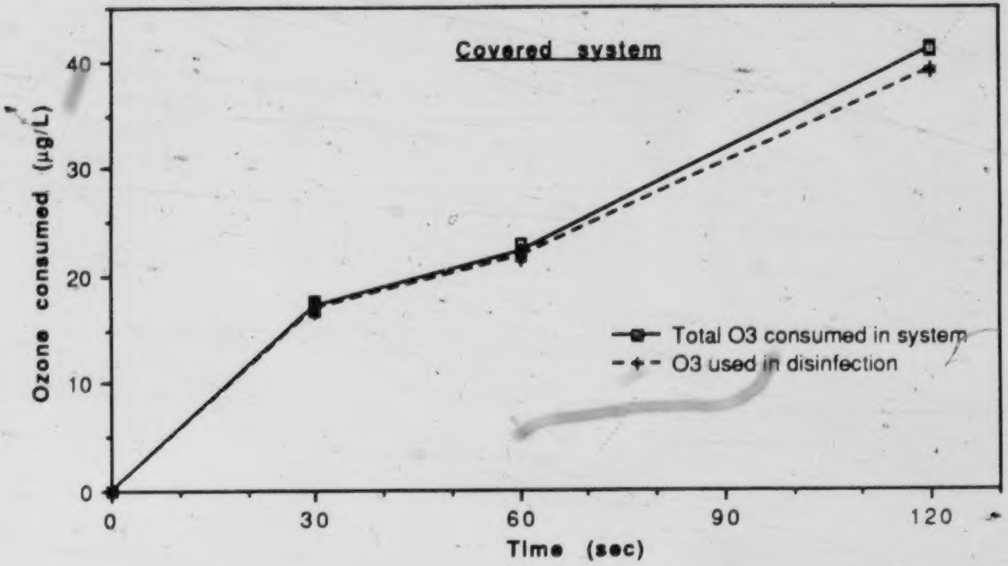


Fig. 4.26

OZONE CONSUMPTION IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING E. coli (No~10^9.3 CFU/dL, Co~46 μg/L, pH 6.9)



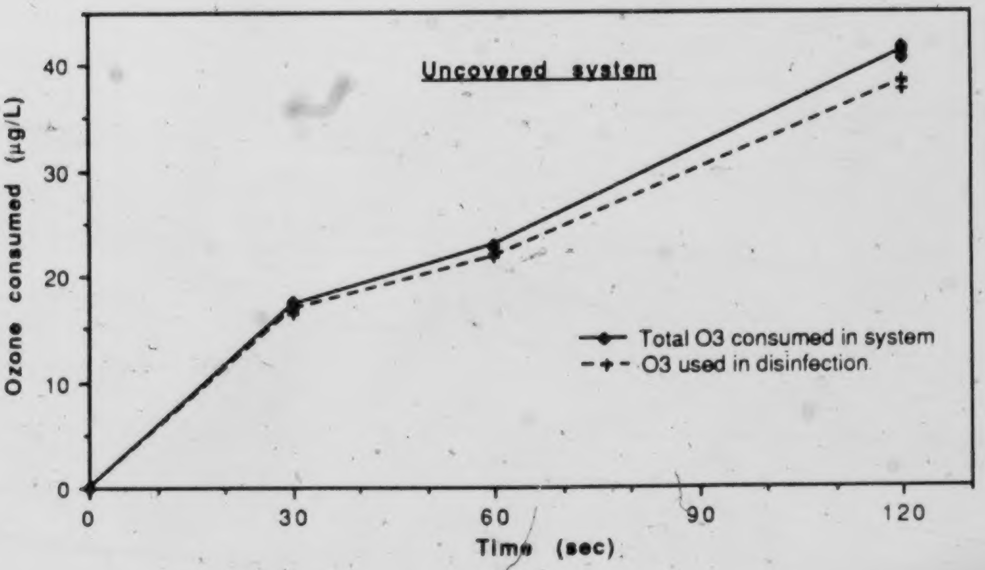
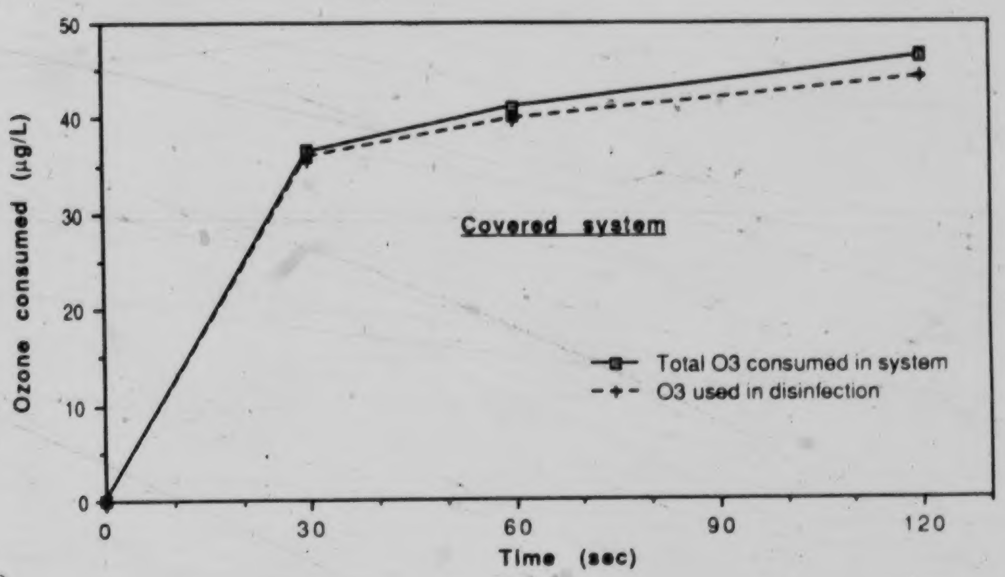


Fig. 4.27

OZONE CONSUMPTION IN 0.05 M PHOSPHATE BUFFER SOLUTION CONTAINING E. coli (No ~ 10^11 CFU/dL, Co~46 μg/L, pH 6.9)



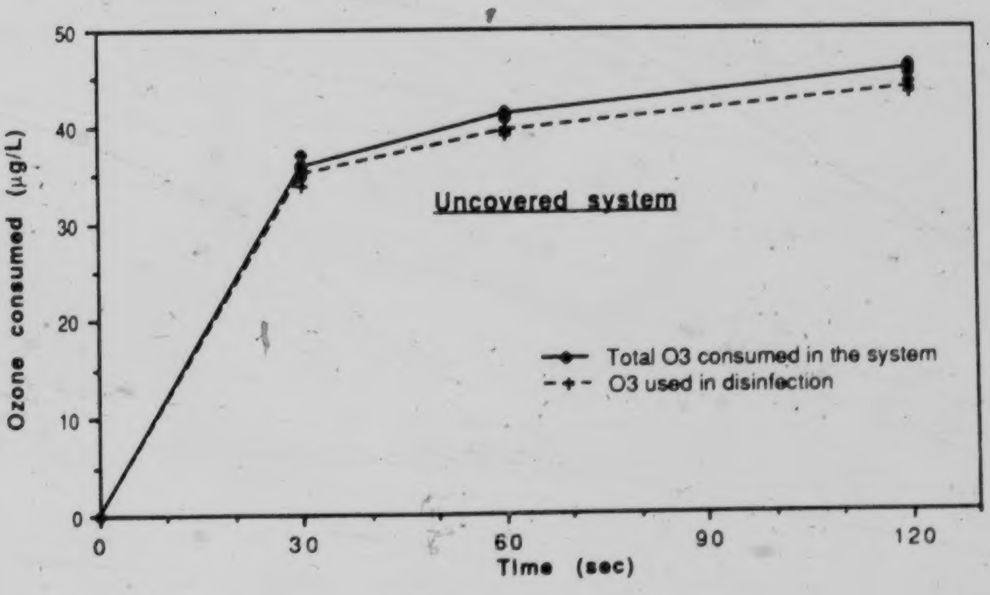
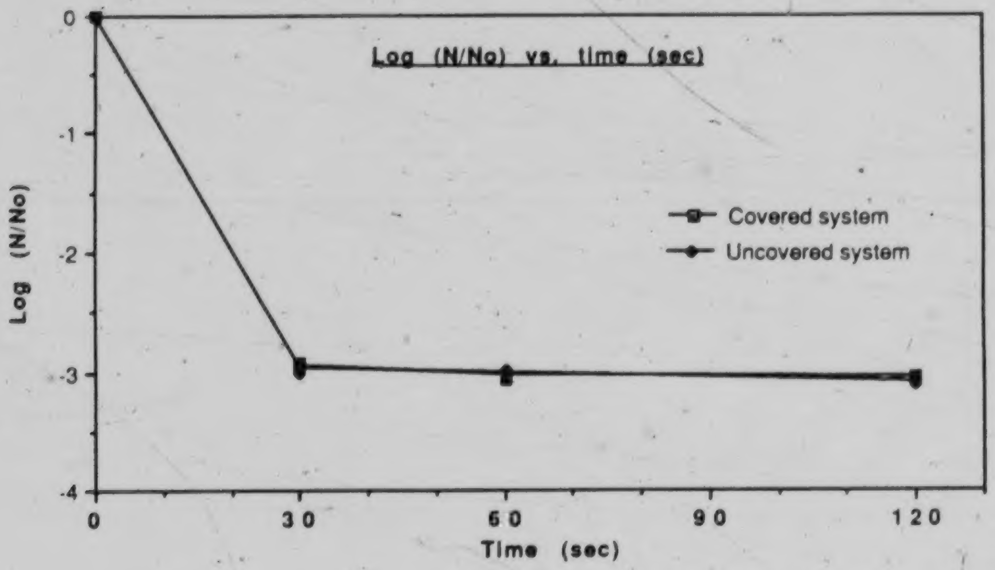


Fig. 4.28

DOSE-RESPONSE IN 0.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^4.2 CFU/dL, Co ~ 46 μg/L, pH 6.9)



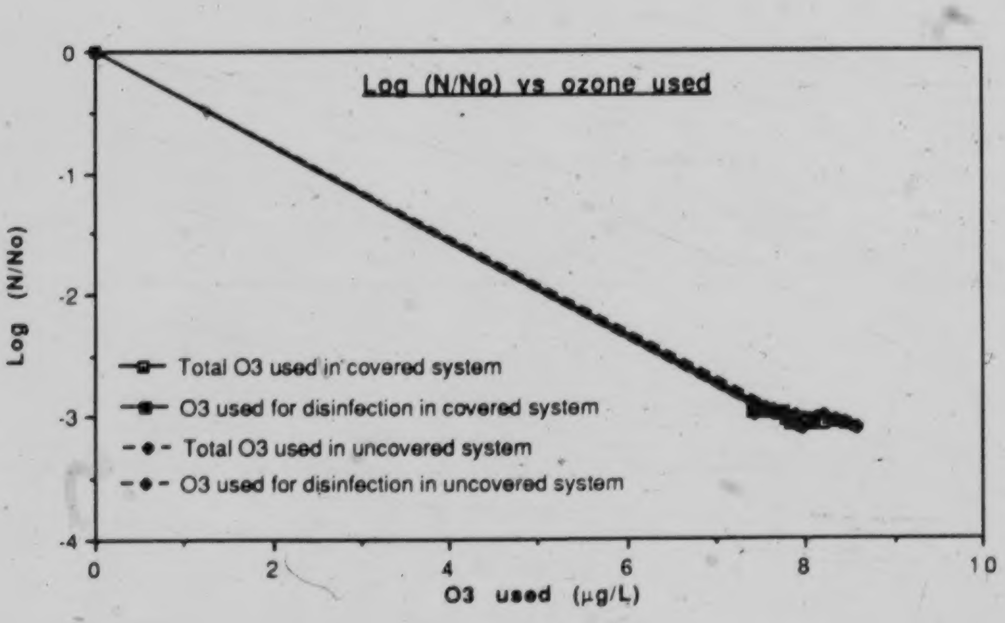


Fig. 4.29

DOSE-RESPONSE IN 0.05 M PHOSPHATE BUFFER SOLUTION
(No ~ 10^7.2 CFU/dL, Co ~ 46 μg/L, pH 6.9)

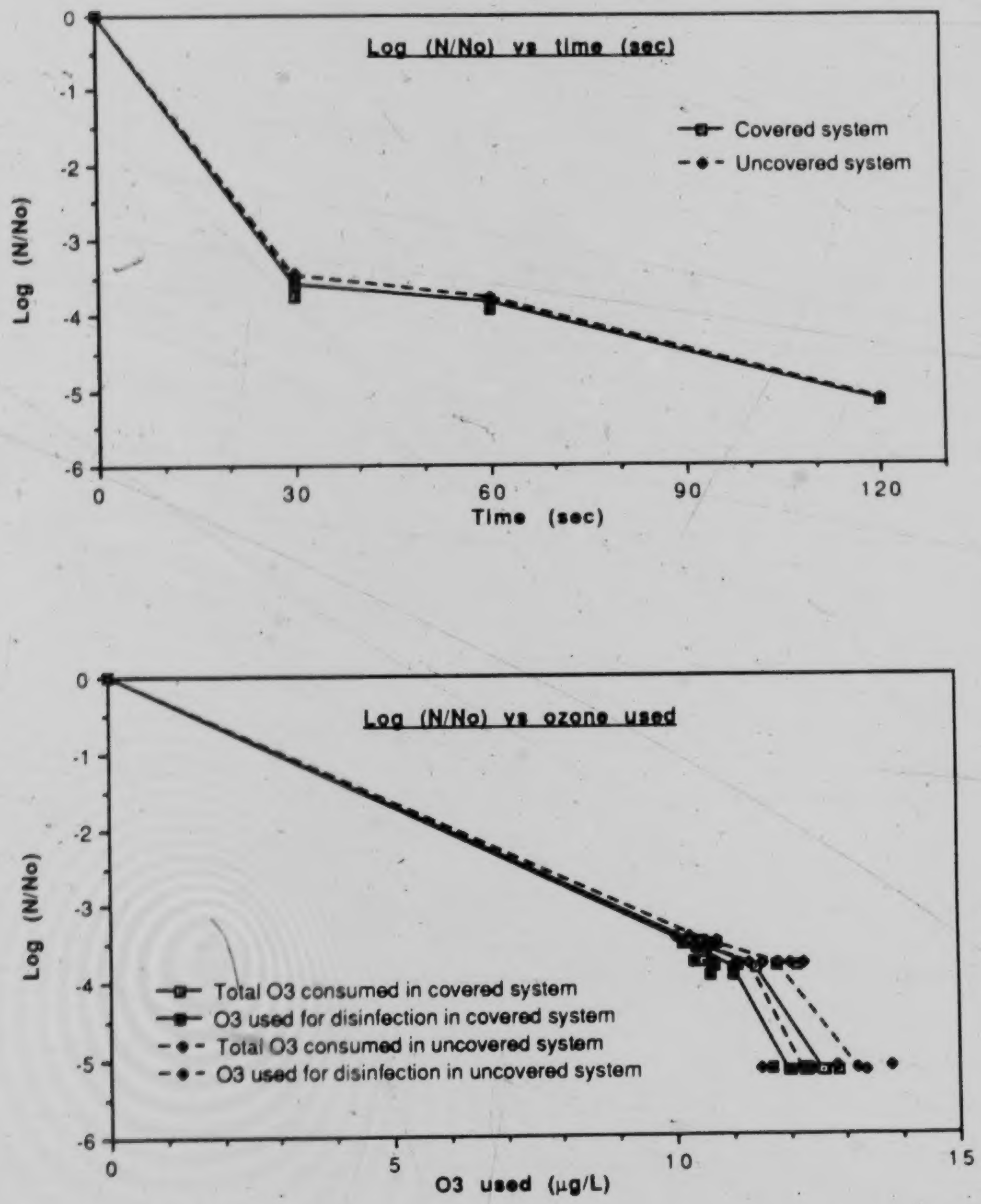
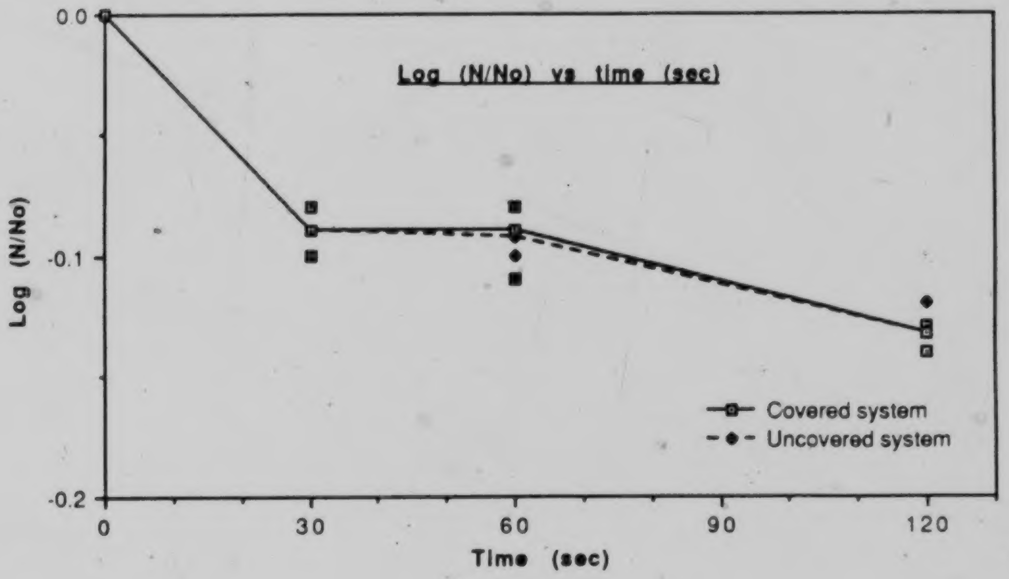


Fig. 4.30

DOSE-RESPONSE IN 0.05 M PHOSPHATE BUFFER SOLUTION (No ~ 10^9.3 CFU/dL, Co ~ 46 μg/L, pH 6.9)



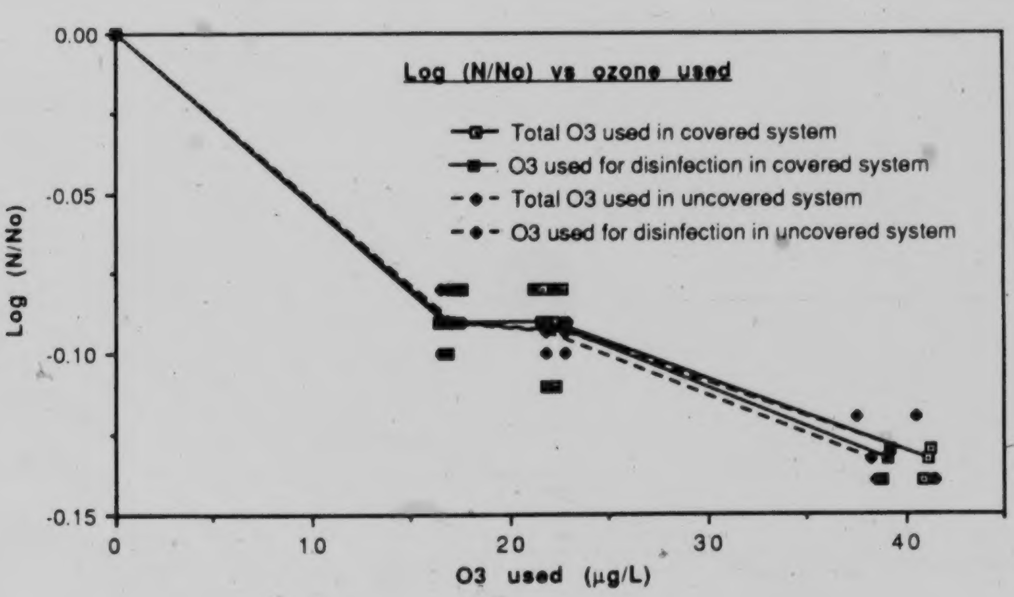
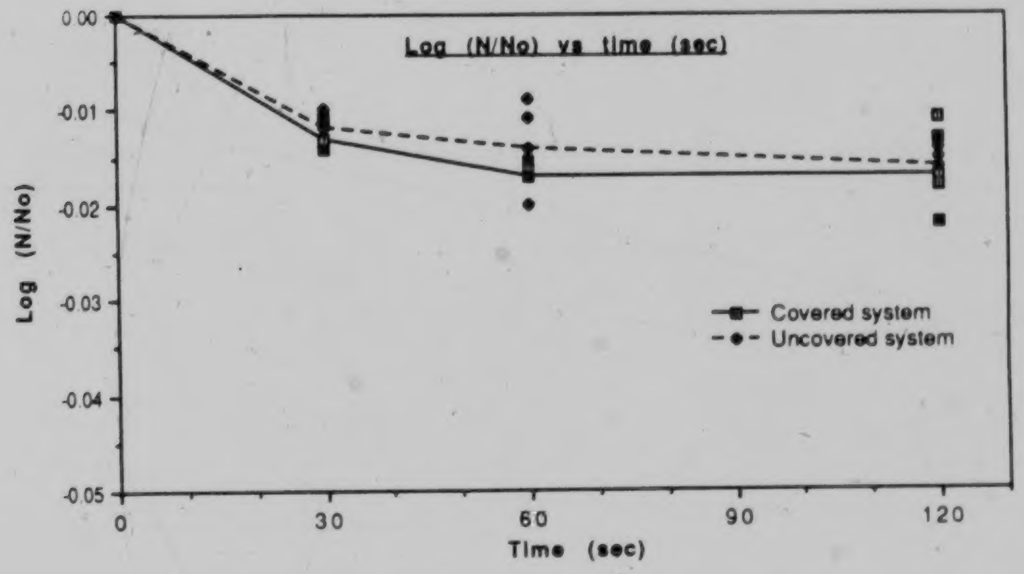
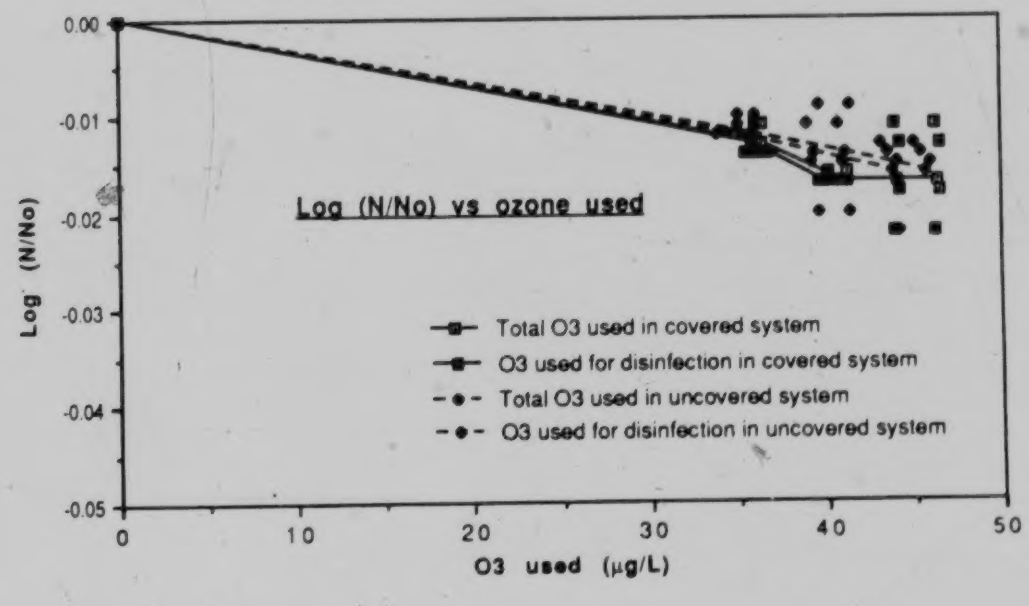


Fig. 4.31

DOSE-RESPONSE IN 0.05 M PHOSPHATE BUFFER SOLUTION
(No ~ 10^11 CFU/dL, Co ~ 46 μg/L, pH 6.9)





- the released material from the lysed cells entrapped the bacteria, so that ozone molecules were not able to reach to the bacterial cells,
- reaction rate constant of ozone with lysed material is much higher than reaction of ozone with *E. coli,* but because the bacterial lysis, in first stage, produces comparatively less amounts of protoplasmic substances, therefore, the quantity of ozone consumption is much smaller than during the first stage.
- The ozone molecules reacted with both but reaction with lysed materials dominated.

Once the quantity of lysed material was reduced to a critical limit, the ozone molecules reacted with both bacteria and lysed material on a competitive basis. Ideally, the reaction rate at this third stage should be somewhere between the first two extremes provided the reactants are at high enough concentrations to make frequent contacts with each other. This phenomena is very clear with $N_o \sim 10^{9.3}$ CFU/dL (Figures 4.26 and 4.30). Unlike other cases, where either ozone consumption or kill took place in two stages; in this case, ozone consumption as well as the disinfection was in three stages. The explanation for this phenomena may be that in other cases, either bacterial cells (with $N_o \sim 10^{4.2}$ CFU/dL and $10^{7.2}$ CFU/dL) or ozone molecules (with 10^{11} CFU/dL) were reduced below a critical level where the proper contacts between cells and molecules could not take place. In this case, on the other hand, even after second stage, the reactants were in high enough concentrations to make frequent contacts with each other and thus both were consumed at a rate which was somewhere in the middle, due the presence of lysed material, of the first two stages.

Ozone consumption was further calculated in terms of ozone molecules utilized to kill one bacterium (Figure 4.32) and was found to be inversely proportional to the bacterial density. For example, the average number of ozone molecules consumed was $10^{7.30}$ to kill one bacterium for an initial bacterial density of 10^{11} CFU/dL, and this number increased to $10^{11.78}$ to kill one bacterium for an initial bacterial density of $10^{4.20}$ CFU/dL. This increase of $10^{4.48}$ times in ozone molecules consumption was not in proportion to the decrease in bacterial densities and thus created a deviation from the linearity. The total ozone molecules used to kill one bacterium after 60 and 120 sec disinfection were also calculated (Figure 4.33) and the difference was compared with those calculated after 30 sec; the difference was not found significant at 1% level.

As discussed earlier, many theories have been developed to explain the phenomena of non-linearity in dose-response relationships but still these explanations are not well established. For example, Chick (1908) and Hom (1972) attributed this deviation from linearity due to the variability of resistance among the cells age distribution of the organisms. Eddy (1953), on the other hand, found no satisfactory evidences that cells in a given culture possessed variable resistance. He, indeed, showed that survivors in a disinfection process gave rise to a population no more resistant than

Fig. 4.32

AVERAGE NUMBER OF OZONE MOLECULES USED PER BACTERIUM REMOVAL FOR DIFFERENT INITIAL E. COLI DENSITIES AT pH 6.9

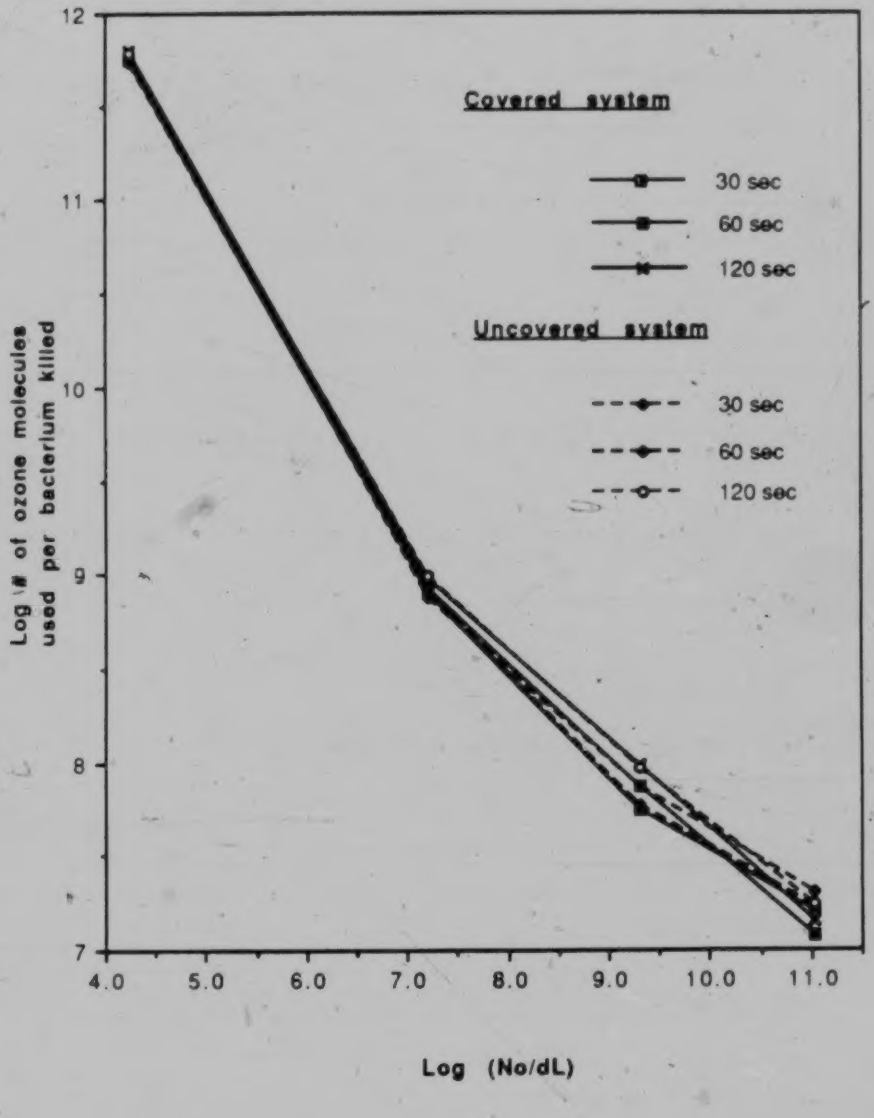
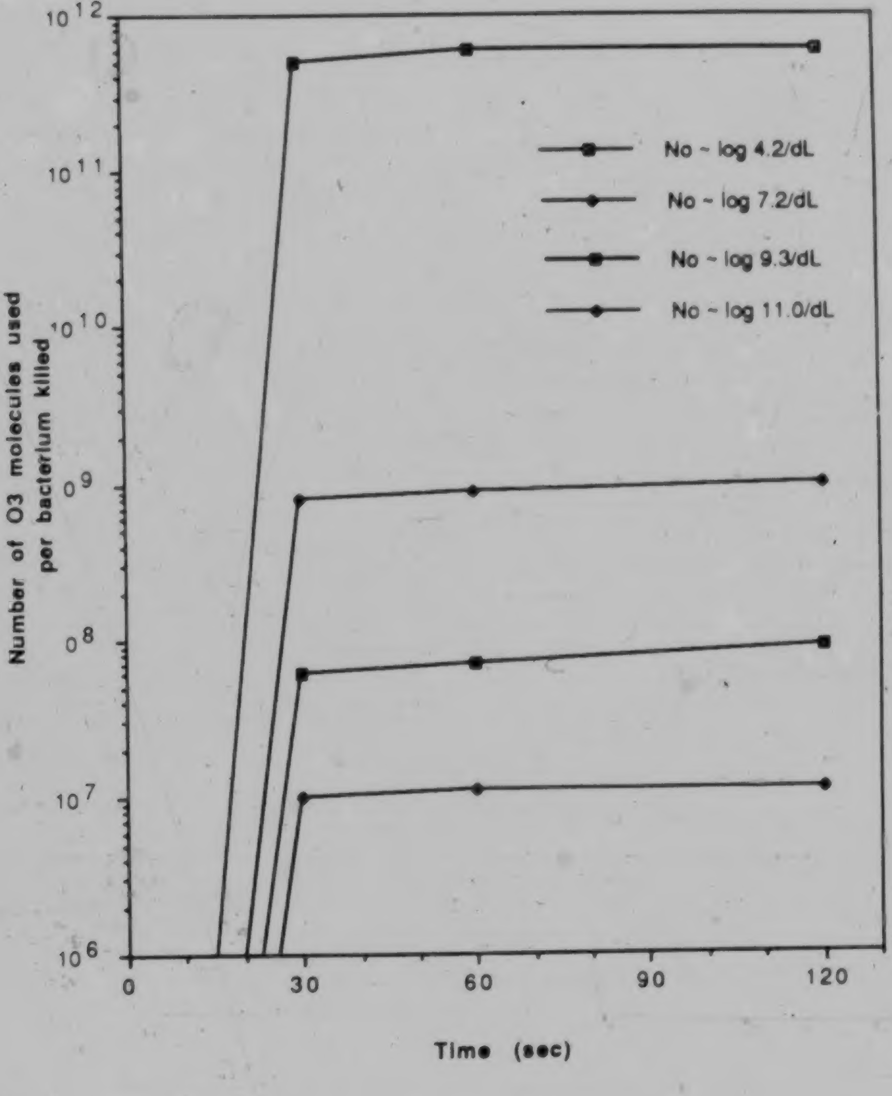


Fig. 4.33

AVERAGE NUMBER OF OZONE MOLECULES VS TIME FOR DIFFERENT INITIAL E. coli DENSITIES (pH 6.9)



the originals. Another possible explanation may be that the lysed material provided protection to the cells from the attack of ozone molecules. This theory satisfies, the lag phase in dose-response experiments, only when enough lysed material is available in the system to react with the ozone molecules. In the beginning, when there is no such cellular debris and, thus, there is no competition for disinfectant, this lag phase should not be there. Presence of bacterial clumps can be quoted as an other possibility. Under same treatment conditions, however, the probability of such clumps is higher at higher bacterial densities, but the results demonstrate that at higher bacterial densities, more kill was achieved. This means that this theory does not satisfy this phenomena either.

The best explanation of this non-linearity can be visualized in terms of the reaction kinetics of the disinfection process and it can be said, as explained earlier, that the disinfection process, in fact, is not a first order reaction with respect to the microorganisms densities.

Comparing the ozone consumption with other studies, Scot and Lesher (1963) using initial *E. coli* density of 10^{11} CFU/dL, determined that since 2.2×10^7 molecules of ozone were consumed in their study to kill one *E. coli* cell, which contains 2.2×10^7 to 2.5×10^7 lipid molecules (Ingraham *et al.*, 1983, Bailey & Ollis, 1986), so probably ozone reacted with the double bonds of the lipids present in the outer membrane. But if it is assumed that ozone molecules reacted with lipid bonds on 1:1 stoichometric bases, not less than 2.2×10^7 ozone molecules are required to kill one bacteria.

In the present study, however, the number of ozone molecules utilized to kill one bacterium was 2×10^7 with a very small amount of ozone dose of 46 µg/L. According to the basic concept of reaction kinetics, the reaction rate between two molecules is a function of the concentration of both reactants and can be expressed for this case as:

$$r = d[O_3]/dt = d[\text{viable bacteria}]/dt = -k[O_3]\ [\text{viable bacteria}]$$

Hoigne (1982) suggested that disinfection is a very fast reaction with high reaction rate constant. This means that in the presence of high initial density of bacteria and small ozone dose, the reaction rate was controlled by the limited supply of ozone dose and if the initial concentration of the ozone was high or if the initial bacterial density was increased, the reaction rate could be even faster and more kill could be expected and vice versa. This phenomena was anticipated from the other studies. Finch (1987) determined that 3×10^8 ozone molecules were consumed to kill one bacterium in dose response experiments. He used an initial bacterial density of $10^{7.2}$ CFU/dL and an ozone dose of 45 µg/L. In the present study, the number of ozone molecules utilized to kill one bacterium varied from 6×10^{11} to 2×10^7 as the bacterial density increased from $10^{4.2}$ CFU/dL to 10^{11} CFU/dL (Figure 4.32). This implies that ozone molecules do not react solely with the lipid molecules.

In contrast, if the structural formation and location of polypeptides and proteins, in a gram-negative cell, are considered (Figure 4.34) which are formed by the condensation reaction between the amino group (-NH_2) of one amino acid molecule and the carboxyl group (-COOH) of the other. The difference between these two is that polypeptides are short chain and proteins are long chain condensed amino acids. The bond resulting from condensation reaction is known as peptide bond and has some of the same characteristics as C=C double bond does (Bailey and Ollis, 1986).

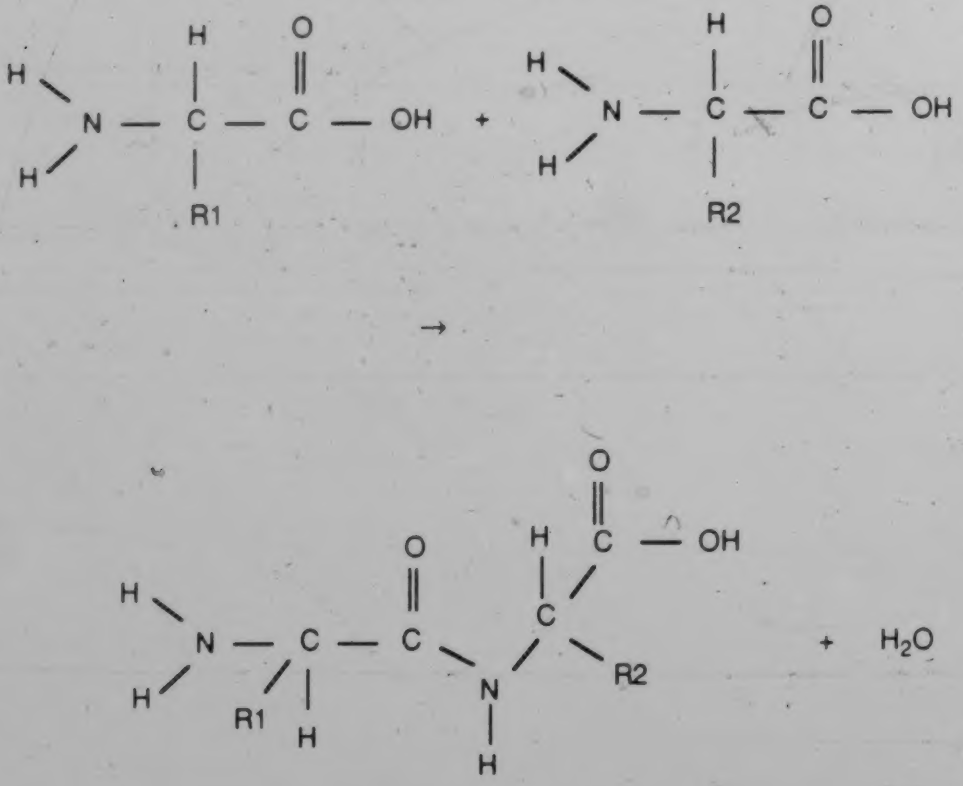


Fig. 4.34 Formation of peptide bond

Because the ozone has a strong reaction potential with the C=C double bonds (Hoigne, 1982) which are located at the outer surface of the outer membrane, therefore proteins and peptides are the first one to come into contact with the ozone molecules (Figure 2.2) and thus susceptible to be oxidized before ozone molecules react with any other constituents of the cell membrane. This direct oxidation of the peptide bonds result in the destruction of the cell's defensive system and, therefore, the ozone molecules penetrate into the periplasmic region and cease or alter the essential enzymes synthetic process. They only enter into the cytoplasmic region of the cell and react with the protoplasm if they are present in large quantities. In principle, this postulation is more close to the findings of Christensen and Giese (1954), Giese and Christensen (1954), Scot and Lesher (1963) and Murray *et al.* (1965).

The data obtained in dose-response experiments was regressed to establish relationships for the kill of microorganisms as a function of independent variables. Because the statistical test on the obtained data showed that no significant difference in the kill at time 30, 60 and 120 sec, therefore, the time was not considered in the regression. The regression analysis, relating *E. coli* kill (N_0-N) in terms of initial bacterial densities and the amount of ozone used in disinfection, yielded following equations:

Model 1:

$$\log (N_o\text{-}N) = -\ 0.187 + 6.17 \log (O_3 \text{ used})$$

$$(s = 0.904,\ R^2 = 79.8\%,\ \text{significance: yes})$$

Model 2:

$$\log (N_o\text{-}N) = 1.21 + 0.781 \log N_o$$

$$(s = 0.325,\ R^2 = 97.4\%,\ \text{significance: yes})$$

Model 3:

$$\log(N_o\text{-}N) = 1.62 - 1.04 \log(O_3 \text{ used}) + 0.892 \log N_o$$
$$(s = 0.308,\ R^2 = 97.7\%,\ \text{significance: yes})$$

Draper and Smith (1966) have mentioned different methods to guess the best regression model keeping in view the number of independent variables and economics of the experimentation. The judgment of the best model is based on the analysis of s (standard error of estimate or the standard deviation of y about the regression line; s also gives an estimate of population standard deviation), R^2 (square of correlation coefficient; it explains the fraction of the variation in y that is explained by the fitted equation), and the significance of various parameters in the model determined by using the partial F-test. The comparison of s and R^2 values for all the three models indicated that number 3 was the most favourable model because it had smallest s and largest R^2 values.

However, to determine if there was lack of fit in the accepted model, two methods were available: i) by splitting the residuals sum of squares in ANOVA table into sum of squares due to the pure error and sum of squares due to the lack of fit and then comparing the F-ratio, obtained by dividing the mean square due to lack of fit by mean square due to pure error, with a standard F-value at desired level of confidence. ii) by examining the residuals. Even if the model does not show any inadequacy using first method, the examination of residuals is recommended (Draper and Smith, 1966). The residuals can be plotted in a number of ways e.g. against the fitted Y-values or against any other independent variable of the model e.g. X_1, X_2 etc. The essential requirement for a model to show adequacy is that the plot of residuals should not indicate any definite trend. The adequacy of the models was examined by plotting the residuals against fitted Y-values (Figures 4.35 to 4.37). It was found that all the plots indicated lack of adequacy. The shape of the graphs indicated that both linear and quadratic terms needed to be included in the models (Draper and Smith, 1966). On the other hand, Caverson *et al.* (1986) concluded that dose-response relationships in ozone disinfection could be described by a model of general form:

$$Y = \beta_o + \beta_1 X_1 + \beta_2 X_2$$

Therefore, instead of incorporating both linear and quadratic terms in the previous obtained models, attempts were made to obtain new equations using different dependent variable. Instead of using (N_o-N), N was used this time and following equations were obtained in terms of initial bacterial densities and ozone used in killing the microorganisms:

Model 4:

$$\log N = -10.23 + 13.26 \log (O_3 \text{ used})$$
($s = 1.523$, $R^2 = 86.5\%$, significance: yes)

Model 5:

$$\log N = -6.27 + 1.56 \log N_o$$
($s = 1.225$, $R^2 = 91.3\%$, significance: yes)

Fig. 4.35

PLOT OF RESIDUALS VS CALCULATED Y
(model 1)

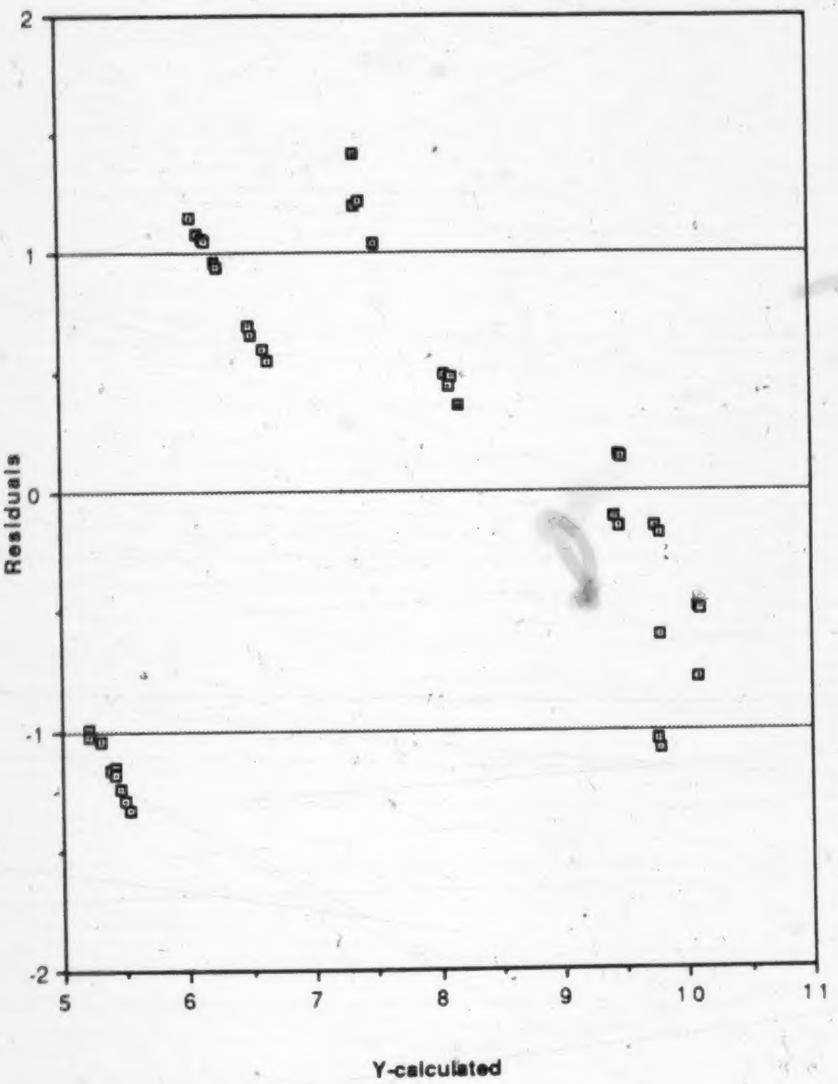


Fig. 4.36

PLOT OF RESIDUALS VS CALCULATED Y
(model 2)

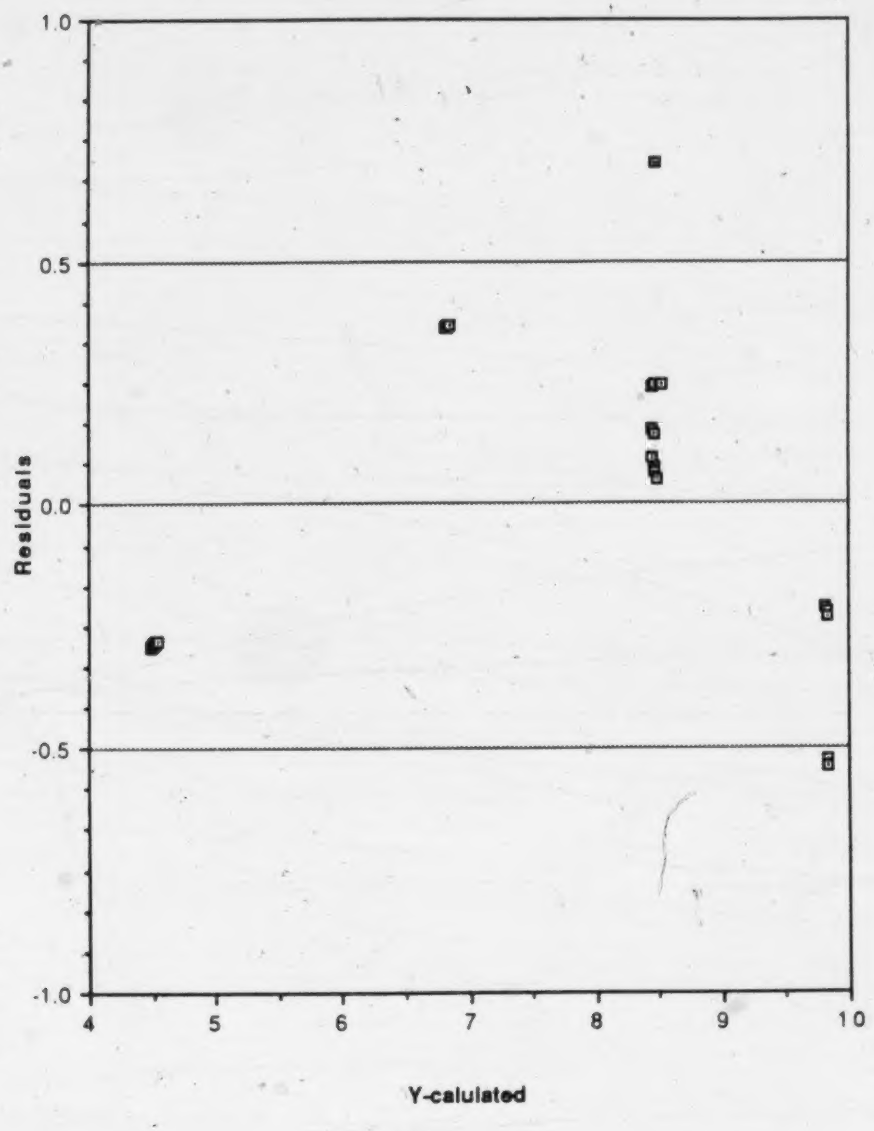
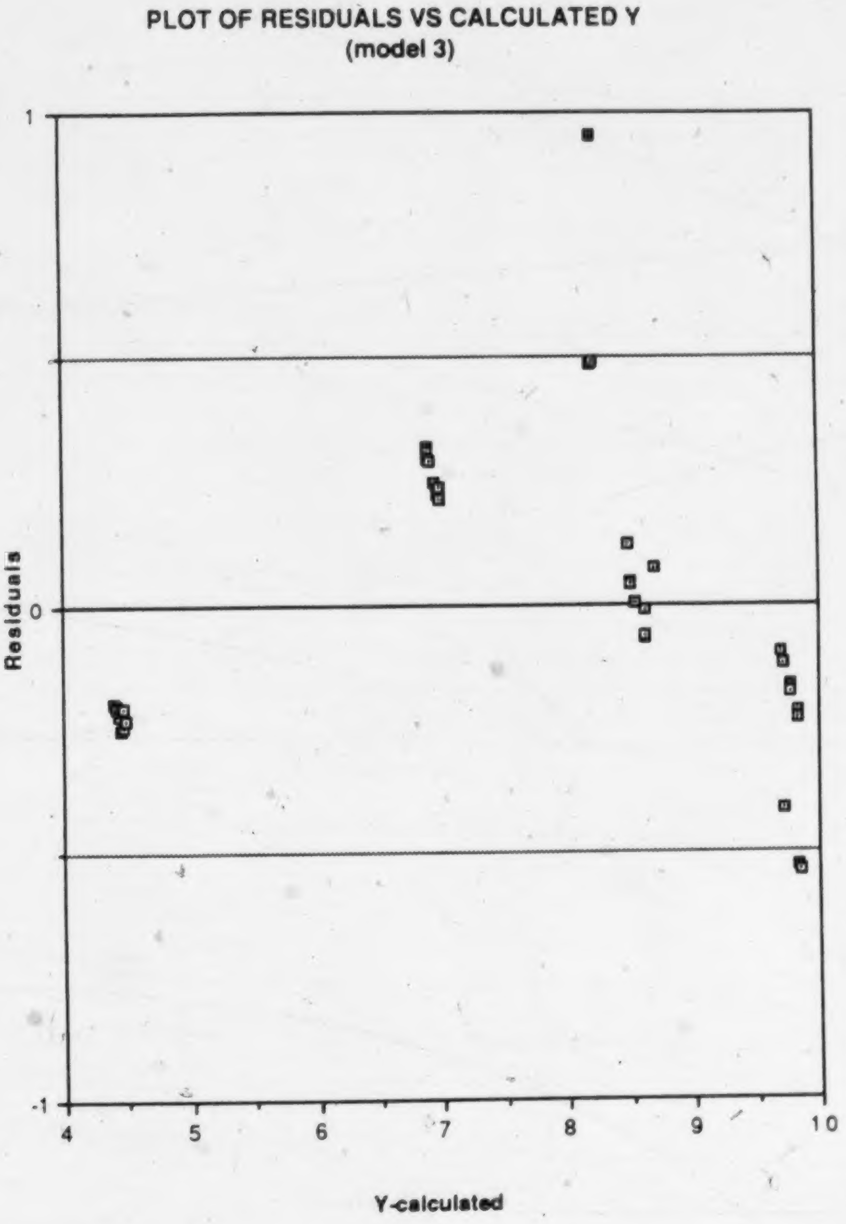


Fig. 4.37


PLOT OF RESIDUALS VS CALCULATED Y
(model 3)
Residuals
1
0
-1
4
5
6
7
8
9
10
Y-calculated

Model 6:

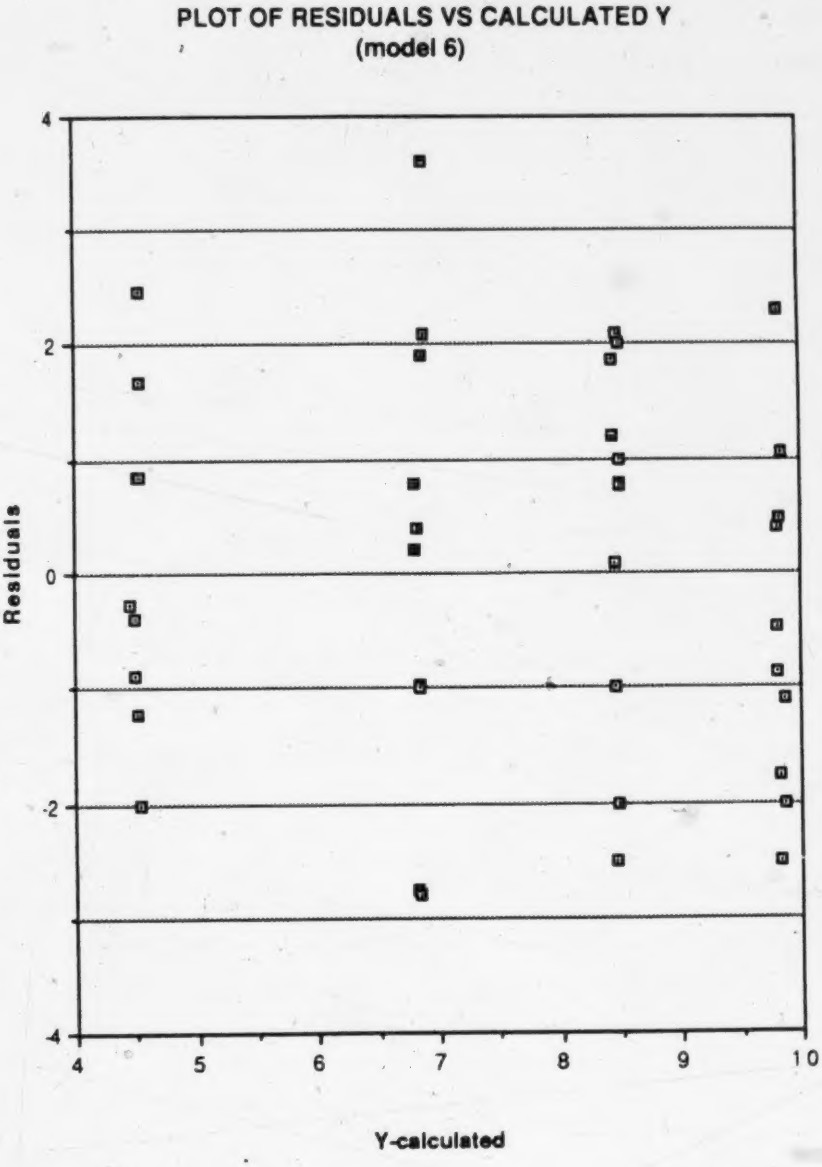
$$\log N = -8.04 - 4.53 \log (O_3 \text{ used}) + 1.08 \log N_o$$
$$(s = 0.308,\ R^2 = 92.7\%,\ \text{significance: yes})$$

Comparison of s and R^2 favoured to choose model 6. The adequacy of this model was also checked by the same procedure as described earlier and it did not show any lack of adequacy (Figure 4.38). It should, however, be pointed out that even if the analysis of residuals establishes the adequacy of the models, it may not be a correct model; and the adequacy test simply reveals that the model has not been found inadequate by the data.

Since both independent variables, N_o and O_3 used, were significant at 95% level for the model 6 and the model did not show any lack of fit, therefore, it was accepted as an equation to describe dose-response relationship in the present study. The 95% confidence limits for this model were as follow:

β_o	$-9.95 \leq \beta_o \leq -6.13$
β_1	$-2.62 \leq \beta_1 \leq -6.44$
β_2	$-0.83 \leq \beta_2 \leq 2.99$

Fig. 4.38


PLOT OF RESIDUALS VS CALCULATED Y
(model 6)
4
2
0
-2
-4
Residuals
4
5
6
7
8
9
10
Y-calculated

5. CONCLUSIONS

From the results obtained in this study, the following specific conclusions are drawn:

1. Ozone disappearance is a function of pH and reaction vessel conditions; however, the amount of ozone which disappears through stripping is insignificant for the equipment used in this study.

2. The auto-decomposition of ozone followed a first order process regardless of the pH of the system and stripping of the ozone to the atmosphere.

3. The pH variations of the 0.05 M phosphate buffer do not exert any adverse effects on the survival of microorganism for up to 60 min.

4. The addition of sodium thiosulphate into the buffer solution gives stability to the bacterial survival up to 30 min detention time.

5. The variations in initial bacterial densities do not affect the survival of suspended organisms for at least up to 60 min.

6. The total ozone consumption was greater in uncovered systems than covered systems, but the difference was not found significant at 5% level.

7. The rate of ozone decomposition is low in acidic regions, therefore, higher concentrations of molecular ozone are available to react with the organisms; the difference in the inactivated *E. coli*, however, was insignificant between kill obtained under acidic or alkaline conditions over the disinfection time applied in this study.

8. 80 to 96% of the total ozone used in the disinfection process was consumed in the first 30 sec. This indicates that the initial attack of ozone is very fast. Consequently, maximum kill is during this phase.

9. It appears from preliminary calculations that OH^o radicals could not be shown to contribute to the disinfection process in these experiments. However, more studies are needed to be under taken to confirm these findings.

10. The bacterial kill is found to be proportional to the initial density of the *E. coli* for a given dose of applied ozone. These findings are in contrast to those of Farooq *et al.* (1977).

11. The ozone disinfection takes place in two distinct stages, depending upon the ozone concentration and the number of viable microorganisms.

12. The individual ozone disinfection stages can be described by first order reaction kinetics but overall disinfection kinetics are essentially not of first order.

13. The effectiveness of the ozone disinfection appears to be limited by the amount of ozone supply.

14. The number of ozone molecules required to inactivate one organism are the same at different pH levels, provided the other system conditions are kept constant (Figure 4.39).

15. The number of ozone molecules utilized to kill one bacterium are inversely proportional to the initial bacterial density. Lower number of ozone molecules are used to disinfect one bacterium as the initial density of *E. coli* increases (Figure 4.39).

16. The number of survived microorganisms was related satisfactorily with ozone used and initial bacterial densities using the following regression equations:

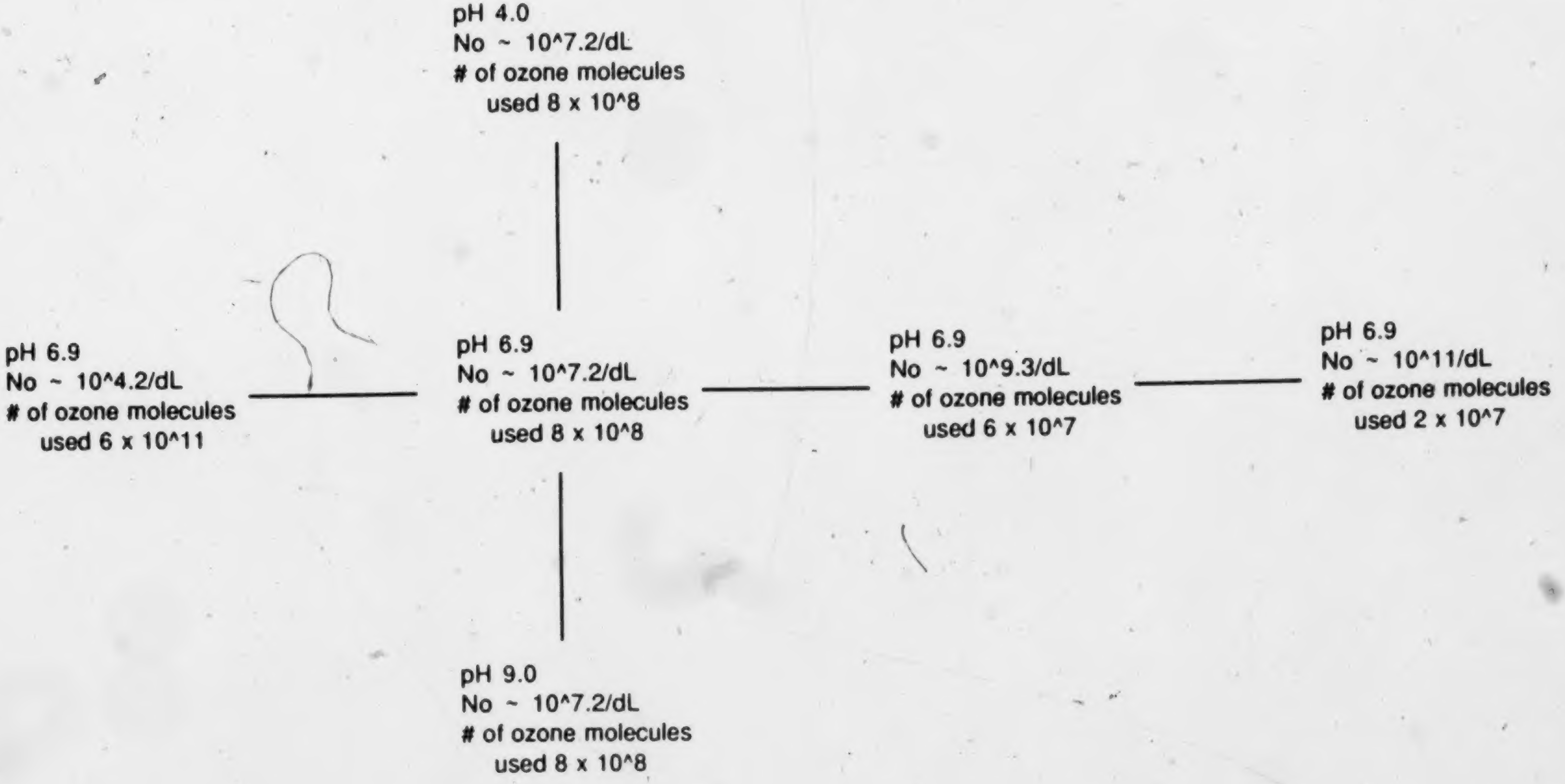
$$\log N = -8.04 - 4.53\,(\log O_3 \text{ used}) + 1.08 \log N_o$$

This equation was found adequate at the following ranges of parameters:

Ozone utilized:	8 to 46 µg/L
N_o	$10^{4.2}$ to 10^{11} CFU/dL
Contact time	30 to 120 sec

Fig. 4.39

NUMBER OF OZONE MOLECULES USED TO KILL ONE BACTERIUM AS A FUNCTION OF pH AND No (in 30 sec)


pH 4.0
No ~ 10^7.2/dL
# of ozone molecules
used 8 x 10^8
pH 6.9
No ~ 10^4.2/dL
# of ozone molecules
used 6 x 10^11
pH 6.9
No ~ 10^7.2/dL
# of ozone molecules
used 8 x 10^8
pH 6.9
No ~ 10^9.3/dL
# of ozone molecules
used 6 x 10^7
pH 6.9
No ~ 10^11/dL
# of ozone molecules
used 2 x 10^7
pH 9.0
No ~ 10^7.2/dL
# of ozone molecules
used 8 x 10^8

6. RECOMMENDATIONS FOR FUTURE WORK

Due to the growing interest in ozone disinfection technology, in water and wastewater treatment, a lot of research is underway now-a-days at advanced levels. It is interesting to know, however, that little basic research has been conducted to understand the effects of various process parameters on the ozone disinfection kinetics. It was, therefore, need of the time that the effects of the parameters, like pH, temperature, initial ozone concentrations, initial bacterial densities, time of disinfection, ozone decomposition, ozone stripping to the atmosphere, presence of different impurities etc., should be investigated on the ozone dose-bacterial response relationships to aid to implement this technology in real world in a better and more efficient way. A part of these investigations has already been presented in this document and it is recommended to address the following areas in future:

1. To confirm the disinfection potential of OH° radicals, the dose-response studies should be conducted using pure OH° radicals in the disinfection system. The combination of hydrogen peroxide and ferrous ions, for example, can be used for this purpose.

2. To understand the ozone disinfection kinetics under various temperature conditions, the efficiency of the process should be investigated at different levels of temperatures.

3. In the disinfection of authentic wastewater, the minimum ozone disinfection time varies from 5 to 10 min, which is longer than the time considered in the present studies. It is, therefore, suggested that the contact time must be extended to observe the disinfection kinetics over longer periods of time.

4. To bring the laboratory scale dose-response kinetics studies more close to the real world situation, the disinfection process should be carried on in the presence of impurities in the system.

5. It is also recommended that the ozone disinfection kinetic studies should be conducted with natural water and wastewater to observe the correlation between the results obtained, under ideal conditions, from laboratory studies and the results obtained from the real situation.

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8. APPENDICIES

Appendix: 1

Preparation of 0.05M phosphate buffer solution

Since

$$H_2PO_4^- = H^+ + HPO_4^{--} \qquad (pKa = 7.2)$$

and

$$C_{T,PO_4^-} = H_2PO_4^- + HPO_4^{--} = 0.05\ M \text{ ------ } (equ.\ 1)$$

as $pH = pK_a + \log [(HPO_4^{--})/(H_2PO_4^-)]$

or $(HPO_4^{--})/(H_2PO_4^-) = [K_a] / [H+]$ ------- (equ. 2)

1- for pH = 4, from equ. 2,

$$(HPO_4^{--})/(H_2PO_4^-) = 10^{-7.2}/10^{-4} = 6.31 * 10^{-4}$$

-------- (equ. 3)

solving equ. 1 & 3 simultaneously,

Na_2HPO_4 = 0.004 gm/L

KH_2PO_4 = 6.800 gm/L

2- for pH = 6.9, from equ. 2,

$(HPO_4^{--})/(H_2PO_4^{-}) = 0.50$ -------- (equ. 4)

solving equ 1 & 4 simultaneously,

Na_2HPO_4 = 2.366 gm/L

KH_2PO_4 = 4.536 gm/L

3- for pH = 9, from equ. 2,

$(HPO_4^{--})/(H_2PO_4^{-}) = 63.10$ -------- (equ. 5)

solving equ 1 & 5 simultaneously,

Na_2HPO_4 = 6.987 gm/L

KH_2PO_4 = 0.106 gm/L

Appendix 2.1(A)

OZONE DECOMPOSITION IN 0.05M PHOSPHATE BUFFER SOLUTION
(Covered system, pH 4)

Run 1

no.	Time (min)	A (Ao = 0.204)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.031	20.60	1.00	0.00	0.00
2	2	0.037	19.88	0.97	-0.02	0.00
3	5	0.045	18.93	0.92	-0.04	0.00
4	10	0.056	17.62	0.86	-0.07	0.01
5	20	0.076	15.24	0.74	-0.13	0.02
6	30	0.097	12.74	0.62	-0.21	0.03
7	45	0.118	10.24	0.50	-0.30	0.05
8	60	0.137	7.98	0.39	-0.41	0.08
9	120	0.176	3.33	0.16	-0.79	0.25

Run 2

no.	Time (min)	A (Ao = 0.204)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.026	21.19	1.00	0.00	0.00
2	5	0.037	19.88	0.94	-0.03	0.00
3	10	0.049	18.45	0.87	-0.06	0.01
4	20	0.074	15.48	0.73	-0.14	0.02
5	30	0.097	12.74	0.60	-0.22	0.03
6	45	0.112	10.95	0.52	-0.29	0.04
7	60	0.131	8.69	0.41	-0.39	0.07
8	120	0.174	3.57	0.17	-0.77	0.23

Run 3

no.	Time (min)	A (Ao = 0.201)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.025	20.95	1.00	0.00	0.00
2	5	0.037	19.52	0.93	-0.03	0.00
3	10	0.051	17.86	0.85	-0.07	0.01
4	20	0.071	15.48	0.74	-0.13	0.02
5	30	0.095	12.62	0.60	-0.22	0.03
6	45	0.111	10.71	0.51	-0.29	0.05
7	60	0.132	8.21	0.39	-0.41	0.07
8	120	0.171	3.57	0.17	-0.77	0.23

Appendix 2.1(B)

OZONE DECOMPOSITION IN 0.05M PHOSPHATE BUFFER SOLUTION
(Uncovered system, pH 4)

Run 1

no.	Time (min)	A (Ao = 0.200)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.020	21.43	1.00	0.00	0.00
2	5	0.036	19.52	0.91	-0.04	0.00
3	10	0.052	17.62	0.82	-0.09	0.01
4	20	0.078	14.52	0.68	-0.17	0.02
5	30	0.099	12.02	0.56	-0.25	0.04
6	45	0.122	9.29	0.43	-0.36	0.06
7	60	0.142	6.90	0.32	-0.49	0.10

Run 2

no.	Time (min)	A (Ao = 0.199)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.025	20.71	1.00	0.00	0.00
2	5	0.044	18.45	0.89	-0.05	0.01
3	10	0.056	17.02	0.82	-0.09	0.01
4	20	0.082	13.93	0.67	-0.17	0.02
5	30	0.100	11.79	0.57	-0.24	0.04
6	45	0.122	9.17	0.44	-0.35	0.06
7	60	0.141	6.90	0.33	-0.48	0.10

Appendix 2.2(A)

OZONE DECOMPOSITION IN 0.05M PHOSPHATE BUFFER SOLUTION
(Covered system, pH 6.9)

Run 1

no.	Time (min)	A (Ao = 0.202)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.028	20.71	1.00	0.00	0.00
2	5	0.047	18.45	0.89	-0.05	0.01
3	10	0.065	16.31	0.79	-0.10	0.01
4	15	0.080	14.52	0.70	-0.15	0.02
5	20	0.094	12.86	0.62	-0.21	0.03
6	30	0.115	10.36	0.50	-0.30	0.05
7	45	0.141	7.26	0.35	-0.46	0.09
8	60	0.158	5.24	0.25	-0.60	0.14

Run 2

no.	Time (min)	A (Ao = 0.200)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.021	21.31	1.00	0.00	0.00
2	5	0.044	18.57	0.87	-0.06	0.01
3	10	0.062	16.43	0.77	-0.11	0.01
4	15	0.073	15.12	0.71	-0.15	0.02
5	20	0.087	13.45	0.63	-0.20	0.03
6	30	0.112	10.48	0.49	-0.31	0.05
7	45	0.141	7.02	0.33	-0.48	0.10
8	60	0.157	5.12	0.24	-0.62	0.15

Run 3

no.	Time (min)	A (Ao = 0.199)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.021	21.19	1.00	0.00	0.00
2	5	0.041	18.81	0.89	-0.05	0.01
3	10	0.057	16.90	0.80	-0.10	0.01
4	15	0.073	15.00	0.71	-0.15	0.02
5	20	0.087	13.33	0.63	-0.20	0.03
6	30	0.112	10.36	0.49	-0.31	0.05
7	45	0.139	7.14	0.34	-0.47	0.09
8	60	0.155	5.24	0.25	-0.61	0.14

Appendix 2.2(B)

OZONE DECOMPOSITION IN 0.05M PHOSPHATE BUFFER SOLUTION
(Uncovered system, pH 6.9)

Run 1

no.	Time (min)	A (Ao = 0.199)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.024	20.83	1.00	0.00	0.00
2	5	0.050	17.74	0.85	-0.07	0.01
3	10	0.069	15.48	0.74	-0.13	0.02
4	15	0.094	12.50	0.60	-0.22	0.03
5	20	0.108	10.83	0.52	-0.28	0.04
6	30	0.134	7.74	0.37	-0.43	0.08
7	45	0.159	4.76	0.23	-0.64	0.16
8	60	0.175	2.86	0.14	-0.86	0.30

Run 2

no.	Time (min)	A (Ao = 0.202)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.025	21.07	1.00	0.00	0.00
2	5	0.055	17.50	0.83	-0.08	0.01
3	10	0.069	15.83	0.75	-0.12	0.02
4	15	0.094	12.86	0.61	-0.21	0.03
5	20	0.114	10.48	0.50	-0.30	0.05
6	30	0.137	7.74	0.37	-0.44	0.08
7	45	0.163	4.64	0.22	-0.66	0.17
8	60	0.174	3.33	0.16	-0.80	0.25

Run 3

no.	Time (min)	A (Ao = 0.201)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.025	20.95	1.00	0.00	0.00
2	5	0.049	18.10	0.86	-0.06	0.01
3	10	0.069	15.71	0.75	-0.12	0.02
4	15	0.095	12.62	0.60	-0.22	0.03
5	20	0.109	10.95	0.52	-0.28	0.04
6	30	0.139	7.38	0.35	-0.45	0.09
7	45	0.159	5.00	0.24	-0.62	0.15
8	60	0.173	3.33	0.16	-0.80	0.25

Appendix 2.3(A)

OZONE DECOMPOSITION IN 0.05M PHOSPHATE BUFFER SOLUTION

(Covered system, pH 9)

Run 1

no.	Time (min)	A (Ao = 0.202)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.021	21.55	1.00	0.00	0.00
2	2	0.037	19.64	0.91	-0.04	0.00
3	5	0.058	17.14	0.80	-0.10	0.01
4	10	0.082	14.29	0.66	-0.18	0.02
5	20	0.132	8.33	0.39	-0.41	0.07
6	30	0.149	6.31	0.29	-0.53	0.11
7	45	0.173	3.45	[illegible]16	-0.80	0.24

Run 2

no.	Time (min)	A (Ao = 0.199)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.019	21.43	1.00	0.00	0.00
2	2	0.031	20.00	0.93	-0.03	0.00
3	5	0.056	17.02	0.79	-0.10	0.01
4	10	0.083	13.81	0.64	-0.19	0.03
5	20	0.126	8.69	0.41	-0.39	0.07
6	30	0.143	6.67	0.31	-0.51	0.10
7	45	0.176	2.74	0.13	-0.89	0.32

Run 3

no.	Time (min)	A (Ao = 0.201)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.025	20.95	1.00	0.00	0.00
2	2	0.037	19.52	0.93	-0.03	0.00
3	5	0.058	17.02	0.81	-0.09	0.01
4	10	0.087	13.57	0.65	-0.19	0.03
5	20	0.129	8.57	0.41	-0.39	0.07
6	30	0.153	5.71	0.27	-0.56	0.13
7	45	0.175	3.10	0.15	-0.83	0.28

Appendix 2.3(B)

OZONE DECOMPOSITION IN 0.05M PHOSPHATE BUFFER SOLUTION
(Uncovered system, pH 9)

Run 1

no.	Time (min)	A (Ao = 0.200)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.019	21.55	1.00	0.00	0.00
2	2	0.046	18.33	0.85	-0.07	0.01
3	5	0.069	15.60	0.72	-0.14	0.02
4	10	0.103	11.55	0.54	-0.27	0.04
5	20	0.148	6.19	0.29	-0.54	0.12
6	30	0.168	3.81	0.18	-0.75	0.22

Run 2

no.	Time (min)	A (Ao = 0.200)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.019	21.55	1.00	0.00	0.00
2	2	0.042	18.81	0.87	-0.06	0.01
3	5	0.069	15.60	0.72	-0.14	0.02
4	10	0.101	11.79	0.55	-0.26	0.04
5	20	0.147	6.31	0.29	-0.53	0.11
6	30	0.171	3.45	0.16	-0.80	0.24

Run 3

no.	Time (min)	A (Ao = 0.201)	C (mg/L)	C/Co	Log (C/Co)	(1/C)-(1/Co)
1	0	0.014	22.26	1.00	0.00	0.00
2	2	0.038	19.40	0.87	-0.06	0.01
3	5	0.062	16.55	0.74	-0.13	0.02
4	10	0.098	12.26	0.55	-0.26	0.04
5	20	0.150	6.07	0.27	-0.56	0.12
6	30	0.170	3.69	0.17	-0.78	0.23

Appendix 3.1(A)

BACTERIAL DIE-OFF COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 4

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 1	32	28	36	32	2.00E-04	4.00	16.00	1.01	7.202
			71	53	61	61	4.00E-04	9.02	81.33	2.66	7.185
2	0	0 - 2	36	26	30	29	2.00E-04	5.03	25.33	1.75	7.161
			75	55	59	62	4.00E-04	10.58	112.00	3.59	7.193
3	0	0 - 3	35	55	30	39	2.00E-04	13.23	175.00	9.05	7.286
			59	66	58	61	4.00E-04	4.36	19.00	0.62	7.183
4	15	15 - 1	69	62	60	64	4.00E-04	4.73	22.33	0.70	7.201
5	15	15 - 2	41	23	31	31	2.00E-04	9.02	81.33	5.28	7.188
			72	53	69	64	4.00E-04	10.21	104.33	3.26	7.205
6	15	15 - 3	36	32	29	32	2.00E-04	3.51	12.33	0.77	7.207
			72	61	62	65	4.00E-04	6.08	37.00	1.14	7.210
7	30	30 - 1	28	39	41	36	2.00E-04	7.00	49.00	2.76	7.249
			66	56	67	63	4.00E-04	6.08	37.00	1.18	7.196
8	30	30 - 2	36	40	29	35	2.00E-04	5.57	31.00	1.79	7.239
			59	63	67	63	4.00E-04	4.00	16.00	0.51	7.197
9	30	30 - 3	74	60	62	65	4.00E-04	7.57	57.33	1.76	7.211

No.	Time (min)	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
			1	2	3						
10	45	45 - 1	23	34	36	30	2.00E-04	7.00	49.00	3.22	7.182
			70	59	62	64	4.00E-04	5.69	32.33	1.02	7.201
11	45	45 - 2	21	33	37	29	2.00E-04	8.33	69.33	4.70	7.169
			71	52	54	58	4.00E-04	10.44	109.00	3.73	7.164
12	45	45 - 3	30	35	31	32	2.00E-04	2.65	7.00	0.44	7.203
			39	65	67	55	4.00E-04	15.62	244.00	8.81	7.141
13	60	60 - 1	24	40	27	30	2.00E-04	8.50	72.33	4.89	7.170
			54	68	61	61	4.00E-04	7.00	49.00	1.61	7.181
14	60	60 - 2	20	38	33	29	2.00E-04	9.29	86.33	5.90	7.165
			61	53	64	59	4.00E-04	5.69	32.33	1.09	7.170
15	60	60 - 3	24	27	41	30	2.00E-04	9.07	82.33	5.52	7.174
			69	59	55	61	4.00E-04	7.21	52.00	1.71	7.181

Appendix 3.1(B)

BACTERIAL DIE-OFF COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 4

No.	Time (min)	Run Identi-fication no.	Counts/plate 1	Counts/plate 2	Counts/plate 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 1	41	28	29	32	2.00E-04	7.23	52.33	3.25	7.206
2	0	0 - 2	42	27	29	29	2.00E[illegible]	8.14	66.33	4.57	7.161
3	0	0 - 3	41	31	26	32	2.00E-04	7.64	58.33	3.64	7.205
4	15	15 - 1	43 57	26 68	35 69	34 64	2.00E-04 4.00E-04	8.50 6.66	72.33 44.33	4.26 1.38	7.230 7.207
5	15	15 - 2	35 64	39 69	20 62	30 65	2.00E-04 4.00E-04	10.02 3.61	100.33 13.00	6.66 0.40	7.178 7.210
6	15	15 - 3	41 67	28 60	30 61	33 63	2.00E-04 4.00E-04	7.00 3.79	49.00 14.33	3.01 0.46	7.211 7.194
7	30	30 - 1	64	54	62	60	4.00E-04	5.29	28.00	0.94	7.175
8	30	30 - 2	35	36	29	33	2.00E-04	3.79	14.33	0.86	7.220
9	30	30 - 3	25 61	39 69	33 63	32 64	2.00E-04 4.00E-04	7.02 4.16	49.33 17.33	3.10 0.54	7.201 7.206

No.	Time (min)	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
			1	2	3						
10	45	45 - 1	31	35	30	32	2.00E-04	2.65	7.00	0.44	7.203
			56	59	64	60	4.00E-04	4.04	16.33	0.55	7.173
11	45	45 - 2	34	34	28	32	2.00E-04	3.46	12.00	0.75	7.202
			53	69	58	60	4.00E-04	8.19	67.00	2.25	7.173
12	45	45 - 3	31	28	37	32	2.00E-04	4.58	21.00	1.32	7.201
			67	54	63	61	4.00E-04	6.66	44.33	1.45	7.184
13	60	60 - 1	35	34	21	29	2.00E-04	7.81	61.00	4.17	7.165
14	60	60 - 2	32	36	24	30	2.00E-04	6.11	37.33	2.47	7.180
			57	62	69	62	4.00E-04	6.03	36.33	1.16	7.194
15	60	60 - 3	61	56	59	59	4.00E-04	2.52	6.33	0.22	7.166

Appendix 3.2(A)

BACTERIAL DIE-OFF COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 -1 - 1	31	31	34	32	2.00E-04	1.73	3.00	0.19	7.204
			65	62	64	64	4.00E-04	1.53	2.33	0.07	7.202
		0 -1 - 2	29	34	34	32	2.00E-04	2.89	8.33	0.52	7.207
			63	62	66	64	4.00E-04	2.08	4.33	0.14	7.202
2	0	0 - 2 - 1	30	33	33	32	2.00E-04	1.73	3.00	0.19	7.204
			64	65	62	64	4.00E-04	1.53	2.33	0.07	7.202
		0 - 2 - 2	30	35	32	32	2.00E-04	2.52	6.33	0.39	7.208
			59	65	68	64	4.00E-04	4.58	21.00	0.66	7.203
3	15	15 - 1 - 1	32	34	31	32	2.00E-04	1.53	2.33	0.14	7.208
			69	60	63	64	4.00E-04	4.58	21.00	0.66	7.203
		15 - 1 - 2	29	36	31	32	2.00E-04	3.61	13.00	0.82	7.202
			66	59	61	62	4.00E-04	3.61	13.00	0.42	7.190
4	15	15 - 2 - 1	31	37	30	33	2.00E-04	3.79	14.33	0.88	7.211
			67	62	62	64	4.00E-04	2.89	8.33	0.26	7.202
		15 - 2 - 2	30	36	36	34	2.00E-04	3.46	12.00	0.71	7.229
			58	67	69	64	4.00E-04	5.86	34.33	1.06	7.207

No.	Time (min)	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
			1	2	3						
5	30	30 - 1 - 1	34	32	31	32	2.00E-04	1.53	2.33	0.14	7.208
			64	60	64	63	4.00E-04	2.31	5.33	0.17	7.195
		30 - 1 - 2	32	30	32	31	2.00E-04	1.15	1.33	0.09	7.195
			59	61	63	61	4.00E-04	2.00	4.00	0.13	7.183
6	30	30 - 2 - 1	33	29	32	31	2.00E-04	2.08	4.33	0.28	7.194
			60	64	64	63	4.00E-04	2.31	5.33	0.17	7.195
		30 - 2 - 2	31	31	33	32	2.00E-04	1.15	1.33	0.08	7.199
			65	59	66	63	4.00E-04	3.79	14.33	0.45	7.199
7	45	45 - 1 - 1	26	28	36	30	2.00E-04	5.29	28.00	1.89	7.172
			61	59	65	62	4.00E-04	3.06	9.33	0.30	7.188
		45 - 1 - 2	33	29	29	30	2.00E-04	2.31	5.33	0.35	7.180
			62	57	59	59	4.00E-04	2.52	6.33	0.21	7.171
8	45	45 - 2 - 1	31	28	28	29	2.00E-04	1.73	3.00	0.21	7.161
			59	59	62	60	4.00E-04	1.73	3.00	0.10	7.176
		45 - 2 - 2	32	28	29	30	2.00E-04	2.08	4.33	0.29	7.171
			64	56	60	60	4.00E-04	4.00	16.00	0.53	7.175
9	60	60 - 1 - 1	30	26	29	28	2.00E-04	2.08	4.33	0.31	7.150
			59	55	54	56	4.00E-04	2.65	7.00	0.25	7.146
		60 - 1 - 2	28	24	30	27	2.00E-04	3.06	9.33	0.69	7.134
			56	60	55	57	4.00E-04	2.65	7.00	0.25	7.154

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
10	60	60 - 2 - 1	28	25	31	28	2.00E-04	3.00	9.00	0.65	7.144
			61	60	52	58	4.00E-04	4.93	24.33	0.85	7.158
		60 - 2 - 2	27	32	26	28	2.00E-04	3.21	10.33	0.73	7.149
			55	62	56	58	4.00E-04	3.79	14.33	0.50	7.158

Appendix 3.2(B)

BACTERIAL DIE-OFF COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	Counts/plate 2	Counts/plate 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 -1 - 1	31	32	33	32	2.00E-04	1.00	1.00	0.06	7.204
			66	62	63	64	4.00E-04	2.08	4.33	0.14	7.202
		0 -1 - 2	28	35	33	32	2.00E-04	3.61	13.00	0.82	7.202
			68	62	60	63	4.00E-04	4.16	17.33	0.55	7.199
2	0	0 - 2 - 1	30	36	32	29	2.00E-04	3.06	9.33	0.64	7.161
			69	65	63	66	4.00E-04	3.06	9.33	0.28	7.215
		0 - 2 - 2	37	28	29	31	2.00E-04	4.93	24.33	1.57	7.192
			70	61	63	65	4.00E-04	4.73	22.33	0.69	7.208
3	15	15 - 1 - 1	30	35	33	33	2.00E-04	2.52	6.33	0.39	7.212
			70	63	61	65	4.00E-04	4.73	22.33	0.69	7.208
		15 - 1 - 2	30	36	31	32	2.00E-04	3.21	10.33	0.64	7.207
			59	67	62	63	4.00E-04	4.04	16.33	0.52	7.194
4	15	15 - 2 - 1	29	35	35	33	2.00E-04	3.46	12.00	0.73	7.216
			60	69	63	64	4.00E-04	4.58	21.00	0.66	7.203
		15 - 2 - 2	27	40	31	32	2.00E-04	6.66	44.33	2.75	7.207
			60	68	65	64	4.00E-04	4.04	16.33	0.51	7.206

No.	Time (min)	Run Identification no.	Counts/plate 1	Counts/plate 2	Counts/plate 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
5	30	30 - 1 - 1	28	32	32	31	2.00E-04	2.31	5.33	0.35	7.185
			71	62	59	64	4.00E-04	6.24	39.00	1.22	7.203
		30 - 1 - 2	29	32	33	31	2.00E-04	2.08	4.33	0.28	7.194
			61	67	58	62	4.00E-04	4.58	21.00	0.68	7.190
6	30	30 - 2 - 1	30	28	36	31	2.00E-04	4.16	17.33	1.11	7.192
			59	59	68	62	4.00E-04	5.20	27.00	0.87	7.189
		30 - 2 - 2	30	31	33	31	2.00E-04	1.53	2.33	0.15	7.195
			57	62	63	61	4.00E-04	3.21	10.33	0.34	7.180
7	45	45 - 1 - 1	35	32	30	32	2.00E-04	2.52	6.33	0.39	7.208
			55	67	63	61	4.00E-04	6.11	37.33	1.21	7.187
		45 - 1 - 2	30	28	31	30	2.00E-04	1.53	2.33	0.16	7.171
			58	59	63	60	4.00E-04	2.65	7.00	0.23	7.176
8	45	45 - 2 - 1	27	30	29	29	2.00E-04	1.53	2.33	0.16	7.156
			62	59	64	62	4.00E-04	2.52	6.33	0.21	7.188
		45 - 2 - 2	32	31	27	30	2.00E-04	2.65	7.00	0.47	7.175
			60	60	57	59	4.00E-04	1.73	3.00	0.10	7.169
9	60	60 - 1 - 1	32	31	25	29	2.00E-04	3.79	14.33	0.98	7.164
			62	57	60	60	4.00E-04	2.52	6.33	0.21	7.173
		60 - 1 - 2	36	24	25	28	2.00E-04	6.66	44.33	3.18	7.144
			58	59	60	59	4.00E-04	1.00	1.00	0.03	7.169

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
10	60	60 - 2 - 1	29	30	27	29	2.00E-04	1.53	2.33	0.16	7.156
			62	60	50	57	4.00E-04	6.43	41.33	1.45	7.154
		60 - 2 - 2	30	26	32	29	2.00E-04	3.06	9.33	0.64	7.165
			61	57	56	58	4.00E-04	2.65	7.00	0.24	7.161

Appendix 3.3(A)

BACTERIAL DIE-OFF COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 9

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 1	31	27	34	31	2.00E-04	3.51	12.33	0.81	7.184
			61	64	69	65	4.00E-04	4.04	16.33	0.51	7.208
2	0	0 - 2	26	41	33	33	2.00E-04	7.51	56.33	3.44	7.214
			69	59	57	61	4.00E-04	6.43	41.33	1.35	7.186
3	0	0 - 3	34	22	41	31	2.00E-04	9.61	92.33	5.90	7.195
			62	70	53	61	4.00E-04	8.50	72.33	2.36	7.185
4	15	15 - 1	32	42	26	33	2.00E-04	8.08	65.33	4.00	7.213
			71	60	62	64	4.00E-04	5.86	34.33	1.07	7.205
5	15	15 - 2	37	32	30	33	2.00E-04	3.61	13.00	0.79	7.216
			71	67	59	65	4.00E-04	6.11	37.33	1.14	7.214
6	15	15 - 3	38	20	31	29	2.00E-04	9.07	82.33	5.74	7.156
			67	64	60	64	4.00E-04	3.51	12.33	0.39	7.201
7	30	30 - 1	41	25	31	32	2.00E-04	8.08	65.33	4.13	7.200
			78	56	57	63	4.00E-04	12.42	154.33	4.91	7.197
8	30	30 - 2	27	36	32	31	2.00E-04	4.51	20.33	1.29	7.197
			62	64	67	64	4.00E-04	2.52	6.33	0.20	7.206
9	30	30 - 3	56	63	70	63	4.00E-04	7.00	49.00	1.56	7.195

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
10	45	45 - 1	34	38	21	30	2.00E-04	8.89	79.00	5.26	7.177
			62	58	61	60	4.00E-04	2.08	4.33	0.14	7.178
11	45	45 - 2	56	63	61	60	4.00E-04	3.61	13.00	0.43	7.176
12	45	45 - 3	53	58	72	60	4.00E-04	9.85	97.00	3.21	7.180
13	60	60 - 1	54	65	66	61	4.00E-04	6.66	44.33	1.44	7.186
14	60	60 - 2	31	32	23	28	2.00E-04	4.93	24.33	1.72	7.152
			60	57	69	62	4.00E-04	6.24	39.00	1.26	7.189
15	60	60 - 3	27	39	31	32	2.00E-04	6.11	37.33	2.34	7.204
			53	67	72	63	4.00E-04	9.85	97.00	3.06	7.201

Appendix 3.3(B)

BACTERIAL DIE-OFF COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 9

No.	Time (min)	Run Identification no.	Counts/plate 1	Counts/plate 2	Counts/plate 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 1	69	59	55	61	4.00E-04	7.21	52.00	1.71	7.181
2	0	0 - 2	37	31	28	32	2.00E-04	4.58	21.00	1.32	7.201
			76	62	65	67	4.00E-04	7.37	54.33	1.61	7.227
3	0	0 - 3	24	36	40	33	2.00E-04	8.33	69.33	4.26	7.212
			51	79	71	66	4.00E-04	14.42	208.00	6.31	7.217
4	15	15 - 1	37	31	33	34	2.00E-04	3.06	9.33	0.56	7.225
			61	57	68	62	4.00E-04	5.57	31.00	1.00	7.189
5	15	15 - 2	34	40	25	32	2.00E-04	7.55	57.00	3.52	7.209
			61	53	59	58	4.00E-04	4.16	17.33	0.60	7.158
6	15	15 - 3	32	39	22	30	2.00E-04	8.54	73.00	4.84	7.179
			53	64	75	63	4.00E-04	11.00	121.00	3.82	7.200
7	30	30 - 1	41	32	30	34	2.00E-04	5.86	34.33	2.02	7.231
			54	53	68	58	4.00E-04	8.39	70.33	2.43	7.161
8	30	30 - 2	25	29	39	30	2.00E-04	7.21	52.00	3.41	7.183
			58	63	59	60	4.00E-04	2.65	7.00	0.23	7.176
9	30	30 - 3	27	34	36	32	2.00E-04	4.73	22.33	1.39	7.205
			61	51	68	60	4.00E-04	8.54	73.00	2.45	7.173

No.	Time (min)	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
			1	2	3						
10	45	45 - 1	27	24	37	29	2.00E-04	6.81	46.33	3.21	7.159
			59	54	67	60	4.00E-04	6.56	43.00	1.44	7.174
11	45	45 - 2	36	24	31	30	2.00E-04	6.03	36.33	2.43	7.175
			76	54	49	59	4.00E-04	14.36	206.33	7.04	7.166
12	45	45 - 3	23	31	36	29	2.00E-04	6.56	43.00	2.92	7.169
			60	63	59	61	4.00E-04	2.08	4.33	0.14	7.181
13	60	60 - 1	21	32	36	29	2.00E-04	7.77	60.33	4.17	7.160
			72	56	59	62	4.00E-04	8.50	72.33	2.33	7.190
14	60	60 - 2	34	27	35	32	2.00E-04	4.36	19.00	1.20	7.201
			61	64	53	59	4.00E-04	5.69	32.33	1.09	7.170
15	60	60 - 3	24	27	41	30	2.00E-04	9.07	82.33	5.52	7.174

Appendix 4.1(A)

DOSE-RESPONSE COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 4.0

No.	Run Identification no.	Counts/plate 1	Counts/plate 2	Counts/plate 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No-1	38	28	32	32	2.00E-04	5.03	25.33	1.56	7.210
	No-2	78	57	57	63	4.00E-04	12.12	147.00	4.65	7.199
	N	39	29	28	32	1.00E+00	6.08	37.00	2.34	3.500
2	30-2-No-1	24	40	27	30	2.00E-04	8.50	72.33	4.89	7.170
	No-2	71	64	59	64	4.00E-04	6.03	36.33	1.13	7.207
	N	41	27	29	32	1.00E+00	7.57	57.33	3.61	3.502
3	30-3-No-1	30	39	31	33	2.00E-04	4.93	24.33	1.47	7.219
	No-2	52	59	61	57	4.00E-04	4.73	22.33	0.78	7.155
	N-1	39	30	31	33	1.00E+00	4.93	24.33	1.47	3.520
	N-2	71	53	57	60	2.00E+00	9.45	89.33	2.98	3.476
4	60-1-No	24	34	39	32	2.00E-04	7.64	58.33	3.68	7.200
	N	25	35	32	30	2.00E+00	5.13	26.33	1.73	3.181
5	60-2-No-1	21	38	31	29	2.00E-04	8.54	73.00	5.01	7.163
	No-2	61	64	60	62	4.00E-04	2.08	4.33	0.14	7.188
	N-1	40	31	33	34	2.00E+00	4.73	22.33	1.30	3.236
	N-2	76	69	78	74	5.00E+00	4.73	22.33	0.60	3.172
6	60-3-No	70	67	53	63	4.00E-04	9.07	82.33	2.62	7.196
	N-1	26	20	22	23	1.00E+00	3.06	9.33	0.83	3.353
	N-2	43	32	36	37	2.00E+00	5.57	31.00	1.69	3.264

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		1	2	3						
7	120-1-No-1	24	35	36	31	2.00E-04	6.66	44.33	2.85	7.192
	No-2	56	69	71	65	4.00E-04	8.14	66.33	2.04	7.211
	N	45	39	41	42	5.00E+01	3.06	9.33	0.45	1.920
8	120-2-No	59	76	54	62	4.00E-04	11.53	133.00	4.27	7.193
	N-1	22	25	20	22	2.50E+01	2.52	6.33	0.57	1.949
	N-2	48	50	39	45	5.00E+01	5.86	34.33	1.51	1.958
9	120-3-No-1	34	28	29	30	2.00E-04	3.21	10.33	0.68	7.179
	No-2	76	54	59	62	4.00E-04	11.53	133.00	4.27	7.193
	N-1	24	20	21	22	2.50E+01	2.08	4.33	0.40	1.937
	N-2	46	42	45	44	5.00E+01	2.08	4.33	0.20	1.947

Appendix 4.1(B)

DOSE-RESPONSE COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 4.0

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No-1	43	29	27	32	2.00E-04	8.72	76.00	4.71	7.208
	No-2	82	59	62	67	4.00E-04	12.50	156.33	4.67	7.224
	N	47	39	37	41	1.00E+00	5.29	28.00	1.37	3.610
2	No	62	47	53	54	4.00E-04	7.55	57.00	2.12	7.128
	N-1	21	25	20	22	5.00E-01	2.65	7.00	0.64	3.641
	N-2	36	54	37	42	1.00E+00	10.12	102.33	4.92	3.619
3	30-3-No-1	28	37	30	31	2.00E-04	4.73	22.33	1.42	7.196
	No-2	57	59	64	60	4.00E-04	3.61	13.00	0.43	7.176
	N	39	33	48	40	1.00E+00	7.55	57.00	2.88	3.597
4	60-1-No-1	27	39	40	35	2.00E-04	7.23	52.33	3.01	7.240
	No-2	59	63	70	64	4.00E-04	5.57	31.00	0.97	7.203
	N	39	43	46	43	2.00E+00	3.51	12.33	0.58	3.328
5	No	63	71	54	62	4.00E-04	8.50	72.33	2.32	7.192
	N-1	24	20	21	22	1.00E+00	2.08	4.33	0.40	3.334
	N-2	37	38	44	40	2.00E+00	3.79	14.33	0.72	3.296
6	60-3-No-1	39	31	28	32	2.00E-04	5.69	32.33	2.00	7.209
	No-2	58	56	68	60	4.00E-04	6.43	41.33	1.37	7.179
	N-1	25	30	24	26	1.00E+00	3.21	10.33	0.79	3.418
	N-2	51	37	39	42	2.00E+00	7.57	57.33	2.74	3.321

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
7	120-1-No-1	30	41	31	34	2.00E-04	6.08	37.00	2.20	7.226
	No-2	64	53	61	59	4.00E-04	5.69	32.33	1.09	7.170
	N	46	50	47	48	5.00E+01	2.08	4.33	0.18	1.979
8	120-2-No-1	24	35	41	33	2.00E-04	8.62	74.33	4.57	7.211
	No-2	64	70	62	65	4.00E-04	4.16	17.33	0.53	7.212
	N-1	25	29	28	27	2.50E+01	2.08	4.33	0.32	2.038
	N-2	54	61	56	57	5.00E+01	3.61	13.00	0.46	2.056
9	120-3-No-1	27	41	35	34	2.00E-04	7.02	49.33	2.92	7.228
	No-2	67	56	59	60	4.00E-04	5.69	32.33	1.07	7.180
	N-1	21	37	32	29	2.50E+01	8.19	67.00	4.59	2.067
	N-2	54	51	62	55	5.00E+01	5.69	32.33	1.17	2.045

Appendix 4.2(A)

DOSE-RESPONSE COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		1	2	3						
1	30-1-No-1	34	29	36	33	2.00E-04	3.61	13.00	0.79	7.216
	No-2	72	61	60	64	4.00E-04	6.66	44.33	1.38	7.205
	N-1	25	19	24	23	5.00E-01	3.21	10.33	0.92	3.653
	N-2	51	47	45	48	1.00E+00	3.06	9.33	0.39	3.678
2	30-2-No-1	28	30	34	31	2.00E-04	3.06	9.33	0.61	7.184
	No-2	74	60	62	65	4.00E-04	7.57	57.33	1.76	7.211
	N	54	46	42	47	1.00E+00	6.11	37.33	1.59	3.673
3	30-3-No-1	37	31	27	31	2.00E-04	5.03	25.33	1.61	7.196
	No-2	71	57	62	63	4.00E-04	7.09	50.33	1.60	7.198
	N-1	22	26	20	23	5.00E-01	3.06	9.33	0.83	3.654
	N-2	48	52	50	50	1.00E+00	2.00	4.00	0.16	3.699
4	30-4-No-1	39	32	30	33	2.00E-04	4.73	22.33	1.34	7.223
	No-2	71	59	65	65	4.00E-04	6.00	36.00	1.11	7.210
	N-1	27	22	20	23	5.00E-01	3.61	13.00	1.14	3.659
	N-2	51	53	54	53	1.00E+00	1.53	2.33	0.09	3.721
5	60-1-No-1	36	31	30	32	2.00E-04	3.21	10.33	0.64	7.207
	No-2	77	59	57	64	4.00E-04	11.02	121.33	3.81	7.202
	N	31	21	22	24	1.00E+00	5.51	30.33	2.50	3.385
6	60-2-No	74	62	57	64	4.00E-04	8.74	76.33	2.39	7.204
	N	42	28	39	36	2.00E+00	7.37	54.33	3.04	3.253

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		1	2	3						
7	60-3-No	59	54	78	63	4.00E-04	12.66	160.33	5.10	7.196
	N-1	34	22	21	25	1.00E+00	7.23	52.33	4.18	3.399
	N-2	53	41	39	44	2.00E+00	7.57	57.33	2.61	3.342
8	120-1-No	68	53	71	63	4.00E-04	9.64	93.00	2.93	7.201
	N	46	61	54	53	5.00E+01	7.51	56.33	2.11	2.028
9	120-2-No	75	54	58	62	4.00E-04	11.15	124.33	4.03	7.188
	N-1	29	20	30	26	2.50E+01	5.51	30.33	2.34	2.016
	N-2	61	39	58	52	5.00E+01	11.93	142.33	5.51	2.014
10	120-3-No	78	59	56	64	4.00E-04	11.93	142.33	4.47	7.202
	N-1	26	34	21	26	2.50E+01	6.56	43.00	3.25	2.025
	N-2	47	59	61	55	5.00E+01	7.57	57.33	2.07	2.044

Appendix 4.2(B)

DOSE-RESPONSE COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		1	2	3						
1	30-1-No-1	27	35	35	32	2.00E-04	4.62	21.33	1.33	7.205
	No-2	69	65	70	68	4.00E-04	2.65	7.00	0.21	7.230
	N-1	27	20	22	23	5.00E-01	3.61	13.00	1.14	3.659
	N-2	47	46	53	49	1.00E+00	3.79	14.33	0.59	3.686
2	30-2-No-1	35	25	32	30	2.00E-04	5.13	26.33	1.73	7.181
	No-2	69	64	57	63	4.00E-04	6.03	36.33	1.15	7.198
	N-1	29	41	25	31	5.00E-01	8.33	69.33	4.48	3.792
	N-2	53	60	45	52	1.00E+00	7.51	56.33	2.15	3.719
3	30-3-No-1	29	37	35	33	2.00E-04	4.16	17.33	1.04	7.224
	No-2	72	49	59	59	4.00E-04	11.53	133.00	4.49	7.171
	N	49	55	48	51	1.00E+00	3.79	14.33	0.57	3.704
4	30-4-No-1	32	35	28	32	2.00E-04	3.51	12.33	0.78	7.198
	No-2	67	50	69	61	4.00E-04	10.44	109.00	3.55	7.186
	N-1	29	23	20	24	5.00E-01	4.58	21.00	1.77	3.676
	N-2	48	57	61	55	1.00E+00	6.66	44.33	1.61	3.741
5	60-1-No-1	34	29	32	32	2.00E-04	2.52	6.33	0.40	7.199
	No-2	69	56	64	63	4.00E-04	6.56	43.00	1.37	7.196
	N	36	24	20	26	1.00E+00	8.33	69.33	5.36	3.413
6	60-2-No	61	55	69	61	2.40E-04	7.02	49.33	1.61	7.408
	N	31	21	25	25	1.00E+00	5.03	25.33	2.00	3.404

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
7	60-3-No	64	71	70	68	4.00E-04	3.79	14.33	0.42	7.232
	N-1	36	21	29	28	1.00E+00	7.51	56.33	4.03	3.447
	N-2	62	59	47	56	2.00E+00	7.94	63.00	2.27	3.444
8	120-1-No	49	69	66	61	4.00E-04	10.79	116.33	3.84	7.181
	N	49	58	65	57	5.00E+01	8.02	64.33	2.26	2.057
9	120-2-No	69	57	67	64	4.00E-04	6.43	41.33	1.29	7.205
	N	69	53	52	58	5.00E+01	9.54	91.00	3.16	2.061
10	120-3-No	76	51	69	64	4.00E-04	12.90	166.33	5.16	7.207
	N	31	20	34	28	2.50E+01	7.37	54.33	3.93	2.043

Appendix 4.3(A)

DOSE-RESPONSE COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 9.0

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		1	2	3						
1	30-1-No-1	35	40	27	34	2.00E-04	6.56	43.00	2.56	7.225
	No-2	64	60	65	63	4.00E-04	2.65	7.00	0.22	7.197
	N	38	45	31	38	5.00E-01	7.00	49.00	2.61	3.876
2	30-2-No-1	36	26	42	34	2.00E-04	8.08	65.33	3.84	7.230
	No-2	60	61	68	63	4.00E-04	4.36	19.00	0.60	7.197
	N	80	71	72	74	1.00E+00	4.93	24.33	0.66	3.871
3	No	64	58	59	60	4.00E-04	3.21	10.33	0.34	7.178
	N-1	35	21	28	27	5.00E-01	7.00	49.00	3.58	3.739
	N-2	66	56	55	59	1.00E+00	6.08	37.00	1.26	3.769
4	60-1-No-1	40	26	31	32	2.00E-04	7.09	50.33	3.16	7.202
	No-2	72	69	64	68	4.00E-04	4.04	16.33	0.48	7.232
	N-1	23	27	20	23	5.00E-01	3.51	12.33	1.07	3.666
	N-2	54	51	46	50	1.00E+00	4.04	16.33	0.65	3.701
5	60-2-No-1	32	42	26	33	2.00E-04	8.08	65.33	4.00	7.213
	No-2	76	54	55	61	4.00E-04	12.42	154.33	5.07	7.182
	N	50	39	34	40	1.00E+00	8.19	67.00	3.31	3.607
6	60-3-No-1	41	33	23	31	2.00E-04	9.02	81.33	5.17	7.197
	No-2	59	67	69	65	4.00E-04	5.29	28.00	0.86	7.210
	N-1	21	32	24	25	5.00E-01	5.69	32.33	2.56	3.704
	N-2	56	45	59	53	1.00E+00	7.37	54.33	2.05	3.724

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
7	120-1-No-1	37	40	26	34	2.00E-04	7.37	54.33	3.22	7.227
	No-2	68	72	61	67	4.00E-04	5.57	31.00	0.93	7.223
	N	47	56	41	48	5.00E+01	7.55	57.00	2.39	1.979
8	120-2-No	41	23	35	32	2.00E-04	9.17	84.00	5.24	7.205
	N	61	51	45	52	2.50E+01	8.08	65.33	2.52	2.317
9	120-3-No	76	56	69	66	4.00E-04	10.15	103.00	3.10	7.221
	N	54	56	42	50	2.50E+01	7.57	57.33	2.28	2.303

Appendix 4.3(B)

DOSE-RESPONSE COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 9.0

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No-1	42	29	30	33	2.00E-04	7.23	52.33	3.15	7.220
	No-2	69	49	58	58	4.00E-04	10.02	100.33	3.45	7.162
	N	40	52	38	43	5.00E-01	7.57	57.33	2.67	3.934
2	30-2-No-1	24	39	34	32	2.00E-04	7.64	58.33	3.68	7.200
	No-2	71	59	54	61	4.00E-04	8.74	76.33	2.51	7.183
	N	51	37	40	42	5.00E-01	7.37	54.33	2.57	3.927
3	30-3-No-1	32	41	35	36	2.00E-04	4.58	21.00	1.17	7.253
	No-2	76	56	71	67	4.00E-04	10.41	108.33	3.23	7.225
	N	36	49	41	42	5.00E-01	6.56	43.00	2.06	3.921
4	60-1-No-1	37	29	40	35	2.00E-04	5.69	32.33	1.85	7.243
	No-2	67	79	72	73	4.00E-04	6.03	36.33	1.00	7.258
	N	64	43	49	51	1.00E+00	10.82	117.00	4.56	3.710
5	60-2-No-1	39	30	24	30	2.00E-04	7.55	57.00	3.75	7.182
	No-2	64	59	57	60	4.00E-04	3.61	13.00	0.43	7.176
	N-1	33	26	28	29	5.00E-01	3.61	13.00	0.90	3.761
	N-2	62	45	54	53	1.00E+00	8.50	72.33	2.72	3.726
6	60-3-No-1	37	29	32	33	2.00E-04	4.04	16.33	1.01	7.211
	No-2	71	59	64	64	4.00E-04	6.03	36.33	1.13	7.207
	N-1	37	31	23	30	5.00E-01	7.02	49.33	3.31	3.775
	N-2	64	69	59	64	1.00E+00	5.00	25.00	0.78	3.805

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
7	120-1-No-1	25	38	35	32	2.00E-04	6.81	46.33	2.88	7.206
	No-2	70	82	56	69	4.00E-04	13.01	169.33	4.94	7.234
	N	74	54	53	60	2.50E+01	11.85	140.33	4.71	2.377
8	120-2-No-1	36	37	30	34	2.00E-04	3.79	14.33	0.84	7.233
	No-2	49	67	73	62	4.00E-04	12.49	156.00	5.02	7.191
	N-1	31	21	24	25	1.00E+01	5.13	26.33	2.11	2.398
	N-2	67	79	54	66	2.50E+01	12.50	156.33	4.75	2.421
9	120-3-No-1	37	35	29	33	2.00E-04	4.16	17.33	1.04	7.224
	No-2	79	62	54	64	4.00E-04	12.77	163.00	5.08	7.205
	N	64	53	62	59	2.50E+01	5.86	34.33	1.15	2.376

Appendix 5.1(A)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2/dL, pH 4.0

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	Log (N/No)	Removal/dL (=No-N)
1	30	C	45	10	1.62E+07	3.16E+03	-3.71	1.62E+07
		U	46	10	1.45E+07	4.07E+03	-3.55	1.45E+07
2	30	C	47	11	1.55E+07	3.18E+03	-3.69	1.55E+07
		U	47	11	1.34E+07	4.27E+03	-3.50	1.34E+07
3	30	C	46	10	1.54E+07	3.15E+03	-3.69	1.54E+07
		U	47	11	1.53E+07	3.95E+03	-3.59	1.53E+07
4	60	C	46	10	1.58E+07	1.52E+03	-4.02	1.58E+07
		U	46	11	1.66E+07	2.13E+03	-3.89	1.66E+07
5	60	C	46	13	1.50E+07	1.60E+03	-3.97	1.50E+07
		U	47	13	1.56E+07	2.07E+03	-3.88	1.56E+07
6	60	C	45	12	1.57E+07	2.04E+03	-3.89	1.57E+07
		U	46	13	1.56E+07	2.34E+03	-3.82	1.66E+07
7	120	C	46	12	1.59E+07	8.32E+01	-5.28	1.59E+07
		U	46	13	1.58E+07	9.53E+01	-5.22	1.58E+07
8	120	C	46	13	1.56E+07	8.99E+01	-5.24	1.56E+07
		U	46	13	1.63E+07	1.11E+02	-5.17	1.63E+07
9	120	C	46	13	1.53E+07	8.75E+01	-5.24	1.53E+07
		U	47	13	1.60E+07	1.14E+02	-5.15	1.60E+07

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	8E+08	0	10	7E+08
	9E+08	0	10	9E+08
2	9E+08	0	11	9E+08
	1E+09	0	11	1E+09
3	8E+08	0	10	8E+08
	9E+08	0	11	9E+08
4	8E+08	0	10	8E+08
	8E+08	0	10	8E+08
5	1E+09	0	13	1E+09
	1E+09	0	13	1E+09
6	9E+08	0	11	9E+08
	1E+09	0	12	1E+09
7	1E+09	1	12	9E+08
	1E+09	1	12	1E+09
8	1E+09	1	12	1E+09
	1E+09	1	12	1E+09
9	1E+09	1	12	1E+09
	1E+09	1	13	1E+09

Appendix 5.1(B)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2/dL, pH 4.0

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	Log (N/No)	Removal/dL (=No-N)
1	30	C	45.48	9.77	1.62E+07	3.16E+03	-3.71	1.62E+07
		U	45.84	10.13	1.45E+07	4.07E+03	-3.55	1.45E+07
2	30	C	46.66	10.95	1.55E+07	3.18E+03	-3.69	1.55E+07
		U	46.90	11.19	1.34E+07	4.27E+03	-3.50	1.34E+07
3	30	C	45.90	10.19	1.54E+07	3.15E+03	-3.69	1.54E+07
		U	46.66	10.95	1.53E+07	3.95E+03	-3.59	1.53E+07
4	60	C	46.08	10.37	1.58E+07	1.52E+03	-4.02	1.58E+07
		U	46.42	10.71	1.66E+07	2.13E+03	-3.89	1.66E+07
5	60	C	46.42	13.09	1.50E+07	1.60E+03	-3.97	1.50E+07
		U	46.66	13.33	1.56E+07	2.07E+03	-3.88	1.56E+07
6	60	C	45.12	11.79	1.57E+07	2.04E+03	-3.89	1.57E+07
		U	45.84	12.51	1.56E+07	2.34E+03	-3.82	1.56E+07
7	120	C	45.72	12.39	1.59E+07	8.32E+01	-5.28	1.59E+07
		U	46.30	12.97	1.58E+07	9.53E+01	-5.22	1.58E+07
8	120	C	46.20	12.87	1.56E+07	8.99E+01	-5.24	1.56E+07
		U	46.42	13.09	1.63E+07	1.11E+02	-5.17	1.63E+07
9	120	C	45.96	12.63	1.53E+07	8.75E+01	-5.24	1.53E+07
		U	46.54	13.21	1.60E+07	1.14E+02	-5.15	1.60E+07

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	7.55E+08	0.20	9.57	7.38E+08
	8.78E+08	0.22	9.91	8.57E+08
2	8.86E+08	0.22	10.73	8.66E+08
	1.04E+09	0.25	10.94	1.02E+09
3	8.30E+08	0.21	9.98	8.12E+08
	8.94E+08	0.24	10.71	8.73E+08
4	8.20E+08	0.29	10.08	7.96E+08
	8.09E+08	0.33	10.38	7.82E+08
5	1.09E+09	0.36	12.73	1.06E+09
	1.07E+09	0.41	12.92	1.04E+09
6	9.41E+08	0.32	11.47	9.13E+08
	1.00E+09	0.39	12.12	9.70E+08
7	9.75E+08	0.52	11.87	9.32E+08
	1.03E+09	0.63	12.34	9.78E+08
8	1.03E+09	0.54	12.33	9.89E+08
	1.01E+09	0.64	12.45	9.56E+08
9	1.03E+09	0.53	12.10	9.86E+08
	1.03E+09	0.64	12.57	9.82E+08

Appendix 5.2(A)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2/dL, pH 6.9

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30	C	46	11	1.63E+07	4.63E+03	2.85E-04	-3.55	1.63E+07
		U	46	11	1.51E+07	4.71E+03	3.11E-04	-3.51	1.51E+07
2	30	C	46	10	1.58E+07	4.71E+03	2.99E-04	-3.53	1.58E+07
		U	46	10	1.55E+07	5.70E+03	3.68E-04	-3.43	1.55E+07
3	30	C	46	10	1.57E+07	4.75E+03	3.02E-04	-3.52	1.57E+07
		U	46	10	1.58E+07	5.06E+03	3.21E-04	-3.49	1.58E+07
4	30	C	46	11	1.65E+07	4.90E+03	2.97E-04	-3.53	1.65E+07
		U	46	11	1.56E+07	5.12E+03	3.29E-04	-3.48	1.56E+07
5	60	C	47	11	1.60E+07	2.43E+03	1.51E-04	-3.82	1.60E+07
		U	47	11	1.58E+07	2.59E+03	1.64E-04	-3.79	1.58E+07
6	60	C	46	11	1.60E+07	1.79E+03	1.12E-04	-3.95	1.60E+07
		U	46	12	2.56E+07	2.54E+03	9.91E-05	-4.00	2.56E+07
7	60	C	45	12	1.57E+07	2.35E+03	1.50E-04	-3.83	1.57E+07
		U	45	12	1.71E+07	2.79E+03	1.64E-04	-3.79	1.71E+07
8	120	C	46	13	1.59E+07	1.07E+02	6.71E-06	-5.17	1.59E+07
		U	46	14	1.52E+07	1.14E+02	7.52E-06	-5.12	1.52E+07
9	120	C	46	12	1.54E+07	1.04E+02	6.71E-06	-5.17	1.54E+07
		U	46	12	1.60E+07	1.15E+02	7.18E-06	-5.14	1.60E+07
10	120	C	45	13	1.59E+07	1.08E+02	6.81E-06	-5.17	1.59E+07
		U	45	13	1.61E+07	1.10E+02	6.85E-06	-5.16	1.61E+07

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	8E+08	0	10	8E+08
	9E+08	0	10	9E+08
2	8E+08	0	10	8E+08
	8E+08	0	10	8E+08
3	8E+08	0	10	8E+08
	8E+08	0	10	8E+08
4	8E+08	0	11	8E+08
	9E+08	0	10	8E+08
5	9E+08	0	11	8E+08
	9E+08	0	11	8E+08
6	9E+08	0	11	8E+08
	6E+08	0	12	6E+08
7	1E+09	0	12	9E+08
	9E+08	1	12	8E+08
8	1E+09	1	12	9E+08
	1E+09	1	13	1E+09
9	1E+09	1	12	9E+08
	1E+09	1	11	9E+08
10	1E+09	1	12	1E+09
	1E+09	1	12	1E+09

Appendix 5.2(B)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2/dL, pH 6.9

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30	C	46.32	10.61	1.63E+07	4.63E+03	2.85E-04	-3.55	1.63E+07
		U	46.32	10.61	1.51E+07	4.71E+03	3.11E-04	-3.51	1.51E+07
2	30	C	45.96	10.25	1.58E+07	4.71E+03	2.99E-04	-3.53	1.58E+07
		U	45.96	10.25	1.55E+07	5.70E+03	3.68E-04	-3.43	1.55E+07
3	30	C	46.20	10.49	1.57E+07	4.75E+03	3.02E-04	-3.52	1.57E+07
		U	46.20	10.49	1.58E+07	5.06E+03	3.21E-04	-3.49	1.58E+07
4	30	C	46.42	10.71	1.65E+07	4.90E+03	2.97E-04	-3.53	1.65E+07
		U	46.42	10.71	1.56E+07	5.12E+03	3.29E-04	-3.48	1.56E+07
5	60	C	46.78	11.07	1.60E+07	2.43E+03	1.51E-04	-3.82	1.60E+07
		U	46.78	11.07	1.58E+07	2.59E+03	1.64E-04	-3.79	1.58E+07
6	60	C	45.72	11.01	1.60E+07	1.79E+03	1.12E-04	-3.95	1.60E+07
		U	45.60	12.27	2.56E+07	2.54E+03	9.91E-05	-4.00	2.56E+07
7	60	C	45.48	12.15	1.57E+07	2.35E+03	1.50E-04	-3.83	1.57E+07
		U	45.36	12.03	1.71E+07	2.79E+03	1.64E-04	-3.79	1.71E+07
8	120	C	45.96	12.63	1.59E+07	1.07E+02	6.71E-06	-5.17	1.59E+07
		U	45.96	13.81	1.52E+07	1.14E+02	7.52E-06	-5.12	1.52E+07
9	120	C	45.90	12.27	1.54E+07	1.04E+02	6.71E-06	-5.17	1.54E+07
		U	45.90	12.39	1.60E+07	1.15E+02	7.18E-06	-5.14	1.60E+07
10	120	C	45.24	12.87	1.59E+07	1.08E+02	6.81E-06	-5.17	1.59E+07
		U	45.24	13.33	1.61E+07	1.10E+02	6.85E-06	-5.16	1.61E+07

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	8.18E+08	0.15	10.46	8.05E+08
	8.79E+08	0.28	10.33	8.54E+08
2	8.14E+08	0.14	10.11	8.01E+08
	8.30E+08	0.27	9.98	8.06E+08
3	8.35E+08	0.15	10.34	8.22E+08
	8.34E+08	0.27	10.22	8.10E+08
4	8.15E+08	0.15	10.56	8.01E+08
	8.63E+08	0.28	10.43	8.38E+08
5	8.65E+08	0.29	10.78	8.41E+08
	8.79E+08	0.46	10.61	8.41E+08
6	8.63E+08	0.29	10.72	8.38E+08
	6.01E+08	0.46	11.81	5.77E+08
7	9.70E+08	0.32	11.83	9.42E+08
	8.84E+08	0.51	11.52	8.45E+08
8	9.96E+08	0.61	12.02	9.46E+08
	1.14E+09	0.99	12.82	1.06E+09
9	9.97E+08	0.59	11.68	9.47E+08
	9.68E+08	0.89	11.50	8.97E+08
10	1.01E+09	0.62	12.25	9.62E+08
	1.04E+09	0.96	12.37	9.60E+08

Appendix 5.3(A)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2 CFU/dL, pH 9.0

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	Log (N/No)	Removal/dL (No-N)
1	30	C	46	10	1.63E+07	7.52E+03	-3.34	1.62E+07
		U	46	10	1.55E+07	8.59E+03	-3.26	1.55E+07
2	30	C	47	11	1.63E+07	7.43E+03	-3.34	1.63E+07
		U	47	11	1.56E+07	8.45E+03	-3.27	1.56E+07
3	30	C	46	10	1.51E+07	5.68E+03	-3.42	1.51E+07
		U	46	10	1.73E+07	8.34E+03	-3.32	1.73E+07
4	60	C	46	12	1.65E+07	4.83E+03	-3.53	1.65E+07
		U	46	13	1.78E+07	5.13E+03	-3.54	1.78E+07
5	60	C	46	13	1.58E+07	4.05E+03	-3.59	1.58E+07
		U	46	12	1.51E+07	5.55E+03	-3.44	1.51E+07
6	60	C	45	12	1.60E+07	5.18E+03	-3.49	1.60E+07
		U	46	12	1.62E+07	6.17E+03	-3.42	1.62E+07
7	120	C	45	17	1.68E+07	2.03E+02	-4.92	1.68E+07
		U	45	16	1.66E+07	2.38E+02	-4.84	1.66E+07
8	120	C	44	16	1.60E+07	2.07E+02	-4.89	1.60E+07
		U	45	16	1.63E+07	2.57E+02	-4.80	1.63E+07
9	120	C	45	16	1.66E+07	2.01E+02	-4.92	1.66E+07
		U	45	16	1.64E+07	2.38E+02	-4.84	1.64E+07

Run no.	Total O3 molecules utilized per bacterium removal	O3 decompose during reaction (ug/L), from equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	8E+08	0	10	8E+08
	8E+08	0	10	8E+08
2	8E+08	0	11	8E+08
	9E+08	1	11	9E+08
3	9E+08	0	10	8E+08
	8E+08	0	10	7E+08
4	9E+08	1	12	9E+08
	9E+08	1	12	8E+08
5	1E+09	1	12	1E+09
	1E+09	1	11	9E+08
6	1E+09	1	11	9E+08
	1E+09	1	12	9E+08
7	1E+09	1	15	1E+09
	1E+09	2	14	1E+09
8	1E+09	1	14	1E+09
	1E+09	2	14	1E+09
9	1E+09	1	15	1E+09
	1E+09	2	14	1E+09

Appendix 5.3(B)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2 CFU/dL, pH 9.0

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	Log (N/No)	Removal/dL (No-N)
1	30	C	45.96	10.25	1.63E+07	7.52E+03	-3.34	1.62E+07
		U	46.20	10.49	1.55E+07	8.59E+03	-3.26	1.55E+07
2	30	C	46.78	11.07	1.63E+07	7.43E+03	-3.34	1.63E+07
		U	46.90	11.19	1.56E+07	8.45E+03	-3.27	1.56E+07
3	30	C	45.96	10.25	1.51E+07	5.68E+03	-3.42	1.51E+07
		U	46.20	10.49	1.73E+07	8.34E+03	-3.32	1.73E+07
4	60	C	45.72	12.39	1.65E+07	4.83E+03	-3.53	1.65E+07
		U	45.96	12.63	1.78E+07	5.13E+03	-3.54	1.78E+07
5	60	C	46.20	12.87	1.58E+07	4.05E+03	-3.59	1.58E+07
		U	45.72	12.39	1.51E+07	5.55E+03	-3.44	1.51E+07
6	60	C	45.48	12.15	1.60E+07	5.18E+03	-3.49	1.60E+07
		U	45.72	12.39	1.62E+07	6.17E+03	-3.42	1.62E+07
7	120	C	45.12	16.55	1.68E+07	2.03E+02	-4.92	1.68E+07
		U	44.52	15.95	1.66E+07	2.38E+02	-4.84	1.66E+07
8	120	C	44.28	15.71	1.60E+07	2.07E+02	-4.89	1.60E+07
		U	44.76	16.19	1.63E+07	2.57E+02	-4.80	1.63E+07
9	120	C	45.00	16.43	1.66E+07	2.01E+02	-4.92	1.66E+07
		U	44.76	16.19	1.64E+07	2.38E+02	-4.84	1.64E+07

Run no.	Total O3 molecules utilized per bacterium removal	O3 decompose during reaction (ug/L), from equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	7.91E+08	0.26	9.99	7.69E+08
	8.47E+08	0.47	10.02	8.07E+08
2	8.50E+08	0.28	10.79	8.27E+08
	9.02E+08	0.50	10.69	8.59E+08
3	8.53E+08	0.26	9.99	8.30E+08
	7.59E+08	0.47	10.02	7.23E+08
4	9.42E+08	0.69	11.70	8.88E+08
	8.88E+08	0.90	11.73	8.23E+08
5	1.02E+09	0.69	12.18	9.66E+08
	1.03E+09	0.94	11.45	9.48E+08
6	9.52E+08	0.69	11.46	8.96E+08
	9.60E+08	0.89	11.50	8.89E+08
7	1.24E+09	1.42	15.13	1.13E+09
	1.20E+09	2.03	13.92	1.05E+09
8	1.23E+09	1.35	14.36	1.12E+09
	1.25E+09	2.06	14.13	1.08E+09
9	1.24E+09	1.41	15.02	1.13E+09
	1.24E+09	2.06	14.13	1.08E+09

Appendix 6.1

OH RADICALS PRODUCED IN DOSE-RESPONSE EXPERIMENT
(No ~ 10^4.2 CFU/dL, Co ~ 46 ug/L, pH 6.9)

Run no.	Time (sec)	System	Log (N/No)	O3 decomposed (ug/L)	OH Radicals produced/dL			
					1:1 ratio	Log no. of OH radicals	1:0.5 ratio	Log no. of OH radicals
1	30	C	-2.98	0.11	1.38E+13	13.14	6.90E+12	12.84
		U	-2.95	0.20	2.51E+13	13.40	1.26E+13	13.10
2	30	C	-2.98	0.11	1.38E+13	13.14	6.90E+12	12.84
		U	-3.00	0.20	2.51E+13	13.40	1.26E+13	13.10
3	30	C	-2.93	0.11	1.38E+13	13.14	6.90E+12	12.84
		U	-2.99	0.20	2.51E+13	13.40	1.26E+13	13.10
4	60	C	-3.03	0.21	2.64E+13	13.42	1.32E+13	13.12
		U	-3.01	0.34	4.27E+13	13.63	2.13E+13	13.33
5	60	C	-3.06	0.21	2.64E+13	13.42	1.32E+13	13.12
		U	-3.03	0.35	4.39E+13	13.64	2.20E+13	13.34
6	60	C	-3.02	0.21	2.64E+13	13.42	1.32E+13	13.12
		U	-3.00	0.34	4.27E+13	13.63	2.13E+13	13.33
7	120	C	-3.06	0.39	4.89E+13	13.69	2.45E+13	13.39
		U	-3.11	0.62	7.78E+13	13.89	3.89E+13	13.59
8	120	C	-3.05	0.40	5.02E+13	13.70	2.51E+13	13.40
		U	-3.06	0.61	7.66E+13	13.88	3.83E+13	13.58
9	120	C	-3.07	0.41	5.15E+13	13.71	2.57E+13	13.41
		U	-3.10	0.62	7.78E+13	13.89	3.89E+13	13.59

Appendix 6.2

OH RADICALS PRODUCED IN DOSE-RESPONSE EXPERIMENT
(No ~ 10^7.2 CFU/dL, Co ~ 46 ug/L, pH 6.9)

Run no.	Time (sec)	System	Log(N/No)	O3 decomposed (ug/L)	OH Radicals produced/dL			
					1:1 ratio	Log no. of OH radicals	1:0.5 ratio	Log no. of OH radicals
1	30	C	-3.55	0.15	1.88E+13	13.27	9.41E+12	12.97
		U	-3.51	0.28	3.51E+13	13.55	1.76E+13	13.24
2	30	C	-3.53	0.14	1.76E+13	13.24	8.79E+12	12.94
		U	-3.43	0.27	3.39E+13	13.53	1.69E+13	13.23
3	30	C	-3.52	0.15	1.88E+13	13.27	9.41E+12	12.97
		U	-3.49	0.27	3.39E+13	13.53	1.69E+13	13.23
4	30	C	-3.53	0.15	1.88E+13	13.27	9.41E+12	12.97
		U	-3.48	0.28	3.51E+13	13.55	1.76E+13	13.24
5	60	C	-3.82	0.29	3.64E+13	13.56	1.82E+13	13.26
		U	-3.79	0.46	5.77E+13	13.76	2.89E+13	13.46
6	60	C	-3.95	0.29	3.64E+13	13.56	1.82E+13	13.26
		U	-4.00	0.46	5.77E+13	13.76	2.89E+13	13.46
7	60	C	-3.83	0.32	4.02E+13	13.60	2.01E+13	13.30
		U	-3.79	0.51	6.40E+13	13.81	3.20E+13	13.51
8	120	C	-5.17	0.61	7.66E+13	13.88	3.83E+13	13.58
		U	-5.12	0.99	1.24E+14	14.09	6.21E+13	13.79
9	120	C	-5.17	0.59	7.40E+13	13.87	3.70E+13	13.57
		U	-5.14	0.89	1.12E+14	14.05	5.58E+13	13.75
10	120	C	-5.17	0.62	7.78E+13	13.89	3.89E+13	13.59
		U	-5.16	0.96	1.20E+14	14.08	6.02E+13	13.78

Appendix 6.3

OH RADICALS PRODUCED IN DOSE-RESPONSE EXPERIMENT
(No ~ 10^9.3 CFU/dL, Co ~ 46 ug/L, pH 6.9)

Run no.	Time (sec)	System	Log(N/No)	O3 decomposed (ug/L)	OH Radicals produced/dL			
					1:1 ratio	Log no. of OH radicals	1:0.5 ratio	Log no. of OH radicals
1	30	C	-0.09	0.25	3.14E+13	13.50	1.57E+13	13.20
		U	-0.07	0.44	5.52E+13	13.74	2.76E+13	13.44
2	30	C	-0.09	0.23	2.89E+13	13.46	1.44E+13	13.16
		U	-0.09	0.46	5.77E+13	13.76	2.89E+13	13.46
3	30	C	-0.09	0.23	2.89E+13	13.46	1.44E+13	13.16
		U	-0.09	0.46	5.77E+13	13.76	2.89E+13	13.46
4	30	C	-0.10	0.24	3.01E+13	13.48	1.51E+13	13.18
		U	-0.09	0.44	5.52E+13	13.74	2.76E+13	13.44
5	60	C	-0.08	0.57	7.15E+13	13.85	3.58E+13	13.55
		U	-0.09	0.96	1.20E+14	14.08	6.02E+13	13.78
6	60	C	-0.09	0.56	7.03E+13	13.85	3.51E+13	13.55
		U	-0.08	0.95	1.19E+14	14.08	5.96E+13	13.78
7	60	C	-0.08	0.58	7.28E+13	13.86	3.64E+13	13.56
		U	-0.10	0.96	1.20E+14	14.08	6.02E+13	13.78
8	60	C	-0.11	1.07	1.34E+14	14.13	6.71E+13	13.83
9	120	C	-0.13	1.98	2.48E+14	14.40	1.24E+14	14.09
		U	-0.14	2.98	3.74E+14	14.57	1.87E+14	14.27
10	120	C	-0.14	1.96	2.46E+14	14.39	1.23E+14	14.09
		U	-0.13	2.99	3.75E+14	14.57	1.88E+14	14.27
11	120	C	-0.14	1.98	2.48E+14	14.40	1.24E+14	14.09
		U	-0.12	2.91	3.65E+14	14.56	1.83E+14	14.26

Appendix 6.4

OH RADICALS PRODUCED IN DOSE-RESPONSE EXPERIMENT
(No ~ 10^11 CFU/dL, Co ~ 46 ug/L, pH 6.9)

Run no.	Time (sec)	System	Log(N/No)	O3 decomposed (ug/L)	OH Radicals produced/dL 1:1 ratio	Log no. of OH radicals	1:0.5 ratio	Log no. of OH radicals
1	30	C	-0.011	0.51	6.40E+13	13.81	3.20E+13	13.51
		U	-0.008	0.90	1.13E+14	14.05	5.65E+13	13.75
2	30	C	-0.012	0.50	6.28E+13	13.80	3.14E+13	13.50
		U	-0.011	0.93	1.17E+14	14.07	5.84E+13	13.77
3	30	C	-0.015	0.51	6.40E+13	13.81	3.20E+13	13.51
		U	-0.015	0.96	1.20E+14	14.08	6.02E+13	13.78
4	30	C	-0.010	0.51	6.40E+13	13.81	3.20E+13	13.51
5	60	C	-0.017	1.06	1.33E+14	14.12	6.65E+13	13.82
		U	-0.015	1.72	2.16E+14	14.33	1.08E+14	14.03
6	60	C	-0.018	1.07	1.34E+14	14.13	6.71E+13	13.83
		U	-0.006	1.74	2.18E+14	14.34	1.09E+14	14.04
7	60	C	-0.018	1.07	1.34E+14	14.13	6.71E+13	13.83
		U	-0.011	1.71	2.15E+14	14.33	1.07E+14	14.03
8	60	U	-0.018	1.74	2.18E+14	14.34	1.09E+14	14.04
9	120	C	-0.023	2.22	2.79E+14	14.44	1.39E+14	14.14
		U	-0.019	1.94	2.43E+14	14.39	1.22E+14	14.09
10	120	C	-0.012	2.22	2.79E+14	14.44	1.39E+14	14.14
		U	-0.011	1.91	2.40E+14	14.38	1.20E+14	14.08
11	120	C	-0.018	2.23	2.80E+14	14.45	1.40E+14	14.15
		U	-0.010	1.89	2.37E+14	14.38	1.19E+14	14.07
12	120	U	-0.014	1.93	2.42E+14	14.38	1.21E+14	14.08

Appendix 6.5

OH RADICALS PRODUCED IN DOSE-RESPONSE EXPERIMENT
(No ~ 10^7.2 CFU/dL, Co ~ 46 ug/L, pH 4)

Run no.	Time (sec)	System	Log(N/No)	O3 decomposed (ug/L)	OH Radicals produced/dL			
					1:1 ratio	Log no. of OH radicals	1:0.5 ratio	Log no. of OH radicals
1	30	C	-3.71	0.20	2.51E+13	13.40	1.26E+13	13.10
		U	-3.55	0.22	2.76E+13	13.44	1.38E+13	13.14
2	30	C	-3.69	0.22	2.76E+13	13.44	1.38E+13	13.14
		U	-3.50	0.25	3.14E+13	13.50	1.57E+13	13.20
3	30	C	-3.69	0.21	2.64E+13	13.42	1.32E+13	13.12
		U	-3.59	0.24	3.01E+13	13.48	1.51E+13	13.18
4	60	C	-4.02	0.29	3.64E+13	13.56	1.82E+13	13.26
		U	-3.89	0.33	4.14E+13	13.62	2.07E+13	13.32
5	60	C	-3.97	0.36	4.52E+13	13.65	2.26E+13	13.35
		U	-3.88	0.41	5.15E+13	13.71	2.57E+13	13.41
6	60	C	-3.89	0.32	4.02E+13	13.60	2.01E+13	13.30
		U	-3.82	0.39	4.89E+13	13.69	2.45E+13	13.39
7	120	C	-5.28	0.52	6.53E+13	13.81	3.26E+13	13.51
		U	-5.22	0.63	7.91E+13	13.90	3.95E+13	13.60
8	120	C	-5.24	0.54	6.78E+13	13.83	3.39E+13	13.53
		U	-5.17	0.64	8.03E+13	13.90	4.02E+13	13.60
9	120	C	-5.24	0.53	6.65E+13	13.82	3.33E+13	13.52
		U	-5.15	0.64	8.03E+13	13.90	4.02E+13	13.60

Appendix 6.6

OH RADICALS PRODUCED IN DOSE-RESPONSE EXPERIMENT
(No ~ 10^7.2 CFU/dL, Co ~ 46 ug/L, pH 9)

Run no.	Time (sec)	System	Log(N/No)	O3 decomposed (ug/L)	OH Radicals produced/dL 1:1 ratio	Log no. of OH radicals	1:0.5 ratio	Log no. of OH radicals
1	30	C	-3.34	0.26	3.26E+13	13.51	1.63E+13	13.21
		U	-3.26	0.47	5.90E+13	13.77	2.95E+13	13.47
2	30	C	-3.34	0.28	3.51E+13	13.55	1.76E+13	13.24
		U	-3.27	0.50	6.28E+13	13.80	3.14E+13	13.50
3	30	C	-3.42	0.26	3.26E+13	13.51	1.63E+13	13.21
		U	-3.32	0.47	5.90E+13	13.77	2.95E+13	13.47
4	60	C	-3.53	0.69	8.66E+13	13.94	4.33E+13	13.64
		U	-3.54	0.90	1.13E+14	14.05	5.65E+13	13.75
5	60	C	-3.59	0.69	8.66E+13	13.94	4.33E+13	13.64
		U	-3.44	0.94	1.18E+14	14.07	5.90E+13	13.77
6	60	C	-3.49	0.69	8.66E+13	13.94	4.33E+13	13.64
		U	-3.42	0.89	1.12E+14	14.05	5.58E+13	13.75
7	120	C	-4.92	1.42	1.78E+14	14.25	8.91E+13	13.95
		U	-4.84	2.03	2.55E+14	14.41	1.27E+14	14.11
8	120	C	-4.89	1.35	1.69E+14	14.23	8.47E+13	13.93
		U	-4.80	2.06	2.59E+14	14.41	1.29E+14	14.11
9	120	C	-4.92	1.41	1.77E+14	14.25	8.85E+13	13.95
		U	-4.84	2.06	2.59E+14	14.41	1.29E+14	14.11

Appendix 7.1(A)

BACTERIAL DIE-OFF COUNTS DATA IN COVERED SYSTEM, No ~ 10^4.2 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 1	46	34	42	40	0.25	6.11	37.33	1.85	4.208
2	0	0 - 2	44	40	41	42	0.25	2.08	4.33	0.21	4.221
3	0	0 - 3	46	34	42	40	0.25	6.11	37.33	1.85	4.208
4	15	15 - 1	49	37	41	42	0.25	6.11	37.33	1.78	4.226
5	15	15 - 2	34	35	42	37	0.25	4.36	19.00	1.03	4.168
6	15	15 - 3	39	40	45	41	0.25	3.21	10.33	0.50	4.218
7	30	30 - 1	45	38	40	41	0.25	3.61	13.00	0.64	4.214
8	30	30 - 2	35	46	38	39	0.25	5.69	32.33	1.64	4.198
9	30	30 - 3	52	34	36	40	0.25	9.87	97.33	4.88	4.203
10	45	45 - 1	39	41	44	41	0.25	2.52	6.33	0.31	4.218
11	45	45 - 2	41	43	37	40	0.25	3.06	9.33	0.46	4.207
12	45	45 - 3	48	43	36	42	0.25	6.03	36.33	1.73	4.226
13	60	60 - 1	35	41	41	39	0.25	3.46	12.00	0.62	4.192
14	60	60 - 2	34	39	42	38	0.25	4.04	16.33	0.86	4.184
15	60	60 - 3	37	40	44	37	0.25	3.51	12.33	0.67	4.170

Appendix 7.1(B)

BACTERIAL DIE-OFF COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^4.2 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 1	39	44	42	42	0.25	2.52	6.33	0.30	4.221
2	0	0 - 2	49	38	37	41	0.25	6.66	44.33	2.16	4.215
3	0	0 - 3	29	43	47	39	0.25	9.45	89.33	4.60	4.191
4	15	15 - 1	36	52	40	42	0.25	8.33	69.33	3.29	4.227
5	15	15 - 2	32	39	46	39	0.25	7.00	49.00	2.54	4.188
6	15	15 - 3	35	42	41	39	0.25	3.79	14.33	0.73	4.195
7	30	30 - 1	38	46	41	42	0.25	4.04	16.33	0.79	4.221
8	30	30 - 2	36	41	51	42	0.25	7.64	58.33	2.76	4.228
9	30	30 - 3	43	36	37	39	0.25	3.79	14.33	0.74	4.188
10	45	45 - 1	40	35	42	39	0.25	3.61	13.00	0.67	4.192
11	45	45 - 2	37	41	42	40	0.25	2.65	7.00	0.35	4.203
12	45	45 - 3	32	39	42	37	0.25	5.13	26.33	1.41	4.175
13	60	60 - 1	35	46	40	40	0.25	5.51	30.33	1.51	4.205
14	60	60 - 2	34	46	42	40	0.25	6.11	37.33	1.85	4.208
15	60	60 - 3	34	35	43	37	0.25	4.93	24.33	1.32	4.170

Appendix 7.2(A)

BACTERIAL DIE-OFF COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	Counts/plate 2	Counts/plate 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 -1 - 1	31	31	34	32	2.00E-04	1.73	3.00	0.19	7.204
			65	62	64	64	4.00E-04	1.53	2.33	0.07	7.202
		0 -1 - 2	29	34	34	32	2.00E-04	2.89	8.33	0.52	7.207
			63	62	66	64	4.00E-04	2.08	4.33	0.14	7.202
2	0	0 - 2 - 1	30	33	33	32	2.00E-04	1.73	3.00	0.19	7.204
			64	65	62	64	4.00E-04	1.53	2.33	0.07	7.202
		0 - 2 - 2	30	35	32	32	2.00E-04	2.52	6.33	0.39	7.208
			59	65	68	64	4.00E-04	4.58	21.00	0.66	7.203
3	15	15 - 1 - 1	32	34	31	32	2.00E-04	1.53	2.33	0.14	7.208
			69	60	63	64	4.00E-04	4.58	21.00	0.66	7.203
		15 - 1 - 2	29	36	31	32	2.00E-04	3.61	13.00	0.82	7.202
			66	59	61	62	4.00E-04	3.61	13.00	0.42	7.190
4	15	15 - 2 - 1	31	37	30	33	2.00E-04	3.79	14.33	0.88	7.211
			67	62	62	64	4.00E-04	2.89	8.33	0.26	7.202
		15 - 2 - 2	30	36	36	34	2.00E-04	3.46	12.00	0.71	7.229
			58	67	69	64	4.00E-04	5.86	34.33	1.06	7.207

No.	Time (min)	Run Identi-fication no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
			1	2	3						
5	30	30 - 1 - 1	34	32	31	32	2.00E-04	1.53	2.33	0.14	7.208
			64	60	64	63	4.00E-04	2.31	5.33	0.17	7.195
		30 - 1 - 2	32	30	32	31	2.00E-04	1.15	1.33	0.09	7.195
			59	61	63	61	4.00E-04	2.00	4.00	0.13	7.183
6	30	30 - 2 - 1	33	29	32	31	2.00E-04	2.08	4.33	0.28	7.194
			60	64	64	63	4.00E-04	2.31	5.33	0.17	7.195
		30 - 2 - 2	31	31	33	32	2.00E-04	1.15	1.33	0.08	7.199
			65	59	66	63	4.00E-04	3.79	14.33	0.45	7.199
7	45	45 - 1 - 1	26	28	36	30	2.00E-04	5.29	28.00	1.89	7.172
			61	59	65	62	4.00E-04	3.06	9.33	0.30	7.188
		45 - 1 - 2	33	29	29	30	2.00E-04	2.31	5.33	0.35	7.180
			62	57	59	59	4.00E-04	2.52	6.33	0.21	7.171
8	45	45 - 2 - 1	31	28	28	29	2.00E-04	1.73	3.00	0.21	7.161
			59	59	62	60	4.00E-04	1.73	3.00	0.10	7.176
		45 - 2 - 2	32	28	29	30	2.00E-04	2.08	4.33	0.29	7.171
			64	56	60	60	4.00E-04	4.00	16.00	0.53	7.175
9	60	60 - 1 - 1	30	26	29	28	2.00E-04	2.08	4.33	0.31	7.150
			59	55	54	56	4.00E-04	2.65	7.00	0.25	7.146
		60 - 1 - 2	28	24	30	27	2.00E-04	3.06	9.33	0.69	7.134
			56	60	55	57	4.00E-04	2.65	7.00	0.25	7.154

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
10	60	60 - 2 - 1	28	25	31	28	2.00E-04	3.00	9.00	0.65	7.144
			61	60	52	58	4.00E-04	4.93	24.33	0.85	7.158
		60 - 2 - 2	27	32	26	28	2.00E-04	3.21	10.33	0.73	7.149
			55	62	56	58	4.00E-04	3.79	14.33	0.50	7.158

Appendix 7.2(B)

BACTERIAL DIE-OFF COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 -1 - 1	31	32	33	32	2.00E-04	1.00	1.00	0.06	7.204
			66	62	63	64	4.00E-04	2.08	4.33	0.14	7.202
		0 -1 - 2	28	35	33	32	2.00E-04	3.61	13.00	0.82	7.202
			68	62	60	63	4.00E-04	4.16	17.33	0.55	7.199
2	0	0 - 2 - 1	30	36	32	29	2.00E-04	3.06	9.33	0.64	7.161
			69	65	63	66	4.00E-04	3.06	9.33	0.28	7.215
		0 - 2 - 2	37	28	29	31	2.00E-04	4.93	24.33	1.57	7.192
			70	61	63	65	4.00E-04	4.73	22.33	0.69	7.208
3	15	15 - 1 - 1	30	35	33	33	2.00E-04	2.52	6.33	0.39	7.212
			70	63	61	65	4.00E-04	4.73	22.33	0.69	7.208
		15 - 1 - 2	30	36	31	32	2.00E-04	3.21	10.33	0.64	7.207
			59	67	62	63	4.00E-04	4.04	16.33	0.52	7.194
4	15	15 - 2 - 1	29	35	35	33	2.00E-04	3.46	12.00	0.73	7.216
			60	69	63	64	4.00E-04	4.58	21.00	0.66	7.203
		15 - 2 - 2	27	40	31	32	2.00E-04	6.66	44.33	2.75	7.207
			60	68	65	64	4.00E-04	4.04	16.33	0.51	7.206

No.	Time (min)	Run Identification no.	Counts/plate 1	 2	 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
5	30	30 - 1 - 1	28	32	32	31	2.00E-04	2.31	5.33	0.35	7.185
			71	62	59	64	4.00E-04	6.24	39.00	1.22	7.203
		30 - 1 - 2	29	32	33	31	2.00E-04	2.08	4.33	0.28	7.194
			61	67	58	62	4.00E-04	4.58	21.00	0.68	7.190
6	30	30 - 2 - 1	30	28	36	31	2.00E-04	4.16	17.33	1.11	7.192
			59	59	68	62	4.00E-04	5.20	27.00	0.87	7.189
		30 - 2 - 2	30	31	33	31	2.00E-04	1.53	2.33	0.15	7.195
			57	62	63	61	4.00E-04	3.21	10.33	0.34	7.180
7	45	45 - 1 - 1	35	32	30	32	2.00E-04	2.52	6.33	0.39	7.208
			55	67	63	61	4.00E-04	6.11	37.33	1.21	7.187
		45 - 1 - 2	30	28	31	30	2.00E-04	1.53	2.33	0.16	7.171
			58	59	63	60	4.00E-04	2.65	7.00	0.23	7.176
8	45	45 - 2 - 1	27	30	29	29	2.00E-04	1.53	2.33	0.16	7.156
			62	59	64	62	4.00E-04	2.52	6.33	0.21	7.188
		45 - 2 - 2	32	31	27	30	2.00E-04	2.65	7.00	0.47	7.175
			60	60	57	59	4.00E-04	1.73	3.00	0.10	7.169
9	60	60 - 1 - 1	32	31	25	29	2.00E-04	3.79	14.33	0.98	7.164
			62	57	60	60	4.00E-04	2.52	6.33	0.21	7.173
		60 - 1 - 2	36	24	25	28	2.00E-04	6.66	44.33	3.18	7.144
			58	59	60	59	4.00E-04	1.00	1.00	0.03	7.169

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
10	60	60 - 2 - 1	29	30	27	29	2.00E-04	1.53	2.33	0.16	7.156
			62	60	50	57	4.00E-04	6.43	41.33	1.45	7.154
		60 - 2 - 2	30	26	32	29	2.00E-04	3.06	9.33	0.64	7.165
			61	57	56	58	4.00E-04	2.65	7.00	0.24	7.161

Appendix 7.3(A)

BACTERIAL DIE-OFF COUNTS DATA IN COVERED SYSTEM, No ~ 10^9.3 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 2 - 1	20	25	22	22	1.00E-06	2.52	6.33	0.57	9.347
		0 - 1 - 2	52	41	39	44	2.00E-06	7.00	49.00	2.25	9.339
		0 - 2 - 2	45	34	46	41	2.00E-06	6.66	44.33	2.15	9.315
2	0	0 - 1 -1	22	23	20	22	1.00E-06	1.53	2.33	0.22	9.335
		0 - 1 - 2	52	42	36	43	2.00E-06	8.08	65.33	3.05	9.331
		0 - 2 - 2	35	49	44	42	2.00E-06	7.09	50.33	2.38	9.325
3	0	0 - 1 - 2	37	33	40	37	2.00E-06	3.51	12.33	0.67	9.262
		0 - 2 - 2	37	36	38	37	2.00E-06	1.00	1.00	0.05	9.267
4	15	15 - 1 - 2	41	47	49	46	2.00E-06	4.16	17.33	0.76	9.357
		15 - 2 - 2	39	48	52	46	2.00E-06	6.66	44.33	1.93	9.362
5	15	15 - 2 - 1	24	20	21	22	1.00E-06	2.08	4.33	0.40	9.334
		15 - 1 - 2	43	49	38	43	2.00E-06	5.51	30.33	1.41	9.333
		15 - 2 - 2	41	47	47	45	2.00E-06	3.46	12.00	0.53	9.351
6	15	15 - 1 - 2	33	35	46	38	2.00E-06	7.00	49.00	2.61	9.274
		15 - 2 - 2	45	35	41	40	2.00E-06	5.03	25.33	1.26	9.302

No.	Time (min)	Run Identi-fication no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
			1	2	3						
7	30	30 - 1 - 2	42	35	46	41	2.00E-06	5.57	31.00	1.52	9.309
		30 - 2 - 2	40	41	38	40	2.00E-06	1.53	2.33	0.12	9.297
8	30	30 - 1 - 2	43	42	42	42	2.00E-06	0.58	0.33	0.02	9.326
		30 - 2 - 2	39	44	41	41	2.00E-06	2.52	6.33	0.31	9.315
9	30	30 - 1 - 2	44	35	32	37	2.00E-06	6.24	39.00	2.13	9.263
		30 - 2 - 2	37	39	45	40	2.00E-06	4.16	17.33	0.86	9.303
10	45	45 - 1 - 2	36	46	41	41	2.00E-06	5.00	25.00	1.23	9.310
		45 - 2 - 2	33	39	49	40	2.00E-06	8.08	65.33	3.28	9.299
11	45	45 - 2 - 1	22	22	20	21	1.00E-06	1.15	1.33	0.13	9.329
		45 - 1 - 2	44	41	36	40	2.00E-06	4.04	16.33	0.81	9.303
		45 - 2 - 2	40	34	40	38	2.00E-06	3.46	12.00	0.63	9.278
12	45	45 - 1 - 1	20	20	22	21	1.00E-06	1.15	1.33	0.13	9.315
		45 - 1 - 2	39	42	38	40	2.00E-06	2.08	4.33	0.22	9.297
		45 - 2 - 2	36	39	33	36	2.00E-06	3.00	9.00	0.50	9.254
13	60	60 - 1 - 2	43	32	33	36	2.00E-06	6.08	37.00	2.07	9.251
		60 - 2 - 2	36	39	26	33	2.00E-06	6.81	46.33	2.79	9.220
14	60	60 - 2 - 1	21	20	22	21	1.00E-06	1.00	1.00	0.10	9.322

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		60 - 1 - 2	34	27	35	32	2.00E-06	4.36	19.00	1.20	9.201
		60 - 2 - 2	61	64	53	59	2.00E-06	5.69	32.33	1.09	9.471
15	60	60 - 1 - 2	44	34	40	39	2.00E-06	5.03	25.33	1.30	9.291
		60 - 2 - 2	32	47	31	36	2.00E-06	8.96	80.33	4.46	9.255

Appendix 7.3(B)

BACTERIAL DIE-OFF COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^9.3 CFU/dL, pH 6.9

No.	Time (min)	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	0	0 - 1 - 2	36	33	40	36	2.00E-06	3.51	12.33	0.68	9.258
		0 - 2 - 2	37	36	38	37	2.00E-06	1.00	1.00	0.05	9.267
2	0	0 - 1 -1	24	23	20	22	1.00E-06	2.08	4.33	0.39	9.348
		0 - 2 - 1	22	20	21	21	1.00E-06	1.00	1.00	0.10	9.322
		0 - 1 - 2	45	41	38	41	2.00E-06	3.51	12.33	0.60	9.314
		0 - 2 - 2	47	36	32	38	2.00E-06	7.77	60.33	3.19	9.277
3	0	0 - 1 -1	21	23	22	22	1.00E-06	1.00	1.00	0.09	9.342
		0 - 2 - 1	26	20	21	22	1.00E-06	3.21	10.33	0.93	9.346
		0 - 1 - 2	37	33	40	37	2.00E-06	3.51	12.33	0.67	9.262
		0 - 2 - 2	37	36	38	37	2.00E-06	1.00	1.00	0.05	9.267
4	15	15 - 1 - 2	41	33	37	37	2.00E-06	4.00	16.00	0.87	9.265
		15 - 2 - 2	36	37	39	37	2.00E-06	1.53	2.33	0.13	9.271
5	15	15 - 1 - 1	22	21	21	21	1.00E-06	0.58	0.33	0.03	9.329
		15 - 1 - 2	36	52	37	41	2.00E-06	8.96	80.33	3.91	9.312
		15 - 2 - 2	35	47	44	42	2.00E-06	6.24	39.00	1.87	9.319
6	15	15 - 1 - 2	42	34	41	39	2.00E-06	4.36	19.00	0.98	9.288
		15 - 2 - 2	36	43	40	40	2.00E-06	3.51	12.33	0.62	9.296

No.	Time (min)	Run Identification no.		Counts/plate		Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
			1	2	3						
7	30	30 - 1 - 2	44	30	32	35	2.00E-06	7.57	57.33	3.29	9.241
		30 - 2 - 2	36	42	44	41	2.00E-06	4.16	17.33	0.86	9.307
8	30	30 - 1 - 2	36	47	38	40	2.00E-06	5.86	34.33	1.71	9.302
		30 - 2 - 2	41	42	35	39	2.00E-06	3.79	14.33	0.73	9.292
9	30	30 - 1 - 2	50	39	32	40	2.00E-06	9.07	82.33	4.15	9.297
		30 - 2 - 2	42	43	36	40	2.00E-06	3.79	14.33	0.71	9.303
10	45	45 - 1 - 2	42	39	30	37	2.00E-06	6.24	39.00	2.13	9.263
		45 - 2 - 2	38	35	36	36	2.00E-06	1.53	2.33	0.13	9.259
11	45	45 - 1 - 2	32	41	46	39	2.00E-06	7.09	50.33	2.57	9.293
		45 - 2 - 2	39	35	41	38	2.00E-06	3.06	9.33	0.49	9.282
12	45	45 - 1 - 2	36	37	42	38	2.00E-06	3.21	10.33	0.54	9.282
		45 - 2 - 2	40	35	41	39	2.00E-06	3.21	10.33	0.54	9.285
13	60	60 - 1 - 2	35	44	39	39	2.00E-06	4.51	20.33	1.04	9.292
		60 - 2 - 2	29	42	43	37	2.00E-06	7.81	61.00	3.26	9.272
14	60	60 - 1 - 2	27	32	42	33	2.00E-06	7.64	58.33	3.52	9.219
		60 - 2 - 2	36	41	36	38	2.00E-06	2.89	8.33	0.44	9.274
15	60	60 - 1 - 2	29	33	44	35	2.00E-06	7.77	60.33	3.47	9.240
		60 - 2 - 2	36	27	40	34	2.00E-06	6.66	44.33	2.62	9.229

Appendix 8.1(A)

COUNTS DATA IN COVERED SYSTEM, No ~ 10^4.2 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No	49	40	45	45	2.50E-01	4.51	20.33	0.91	4.251
	N	35	35	43	37	2.00E+02	4.62	21.33	1.14	1.273
2	30-2-No	37	41	47	41	2.50E-01	5.03	25.33	1.22	4.220
	N	39	28	39	35	2.00E+02	6.35	40.33	2.31	1.242
3	30-3-No	40	39	38	39	2.50E-01	1.00	1.00	0.05	4.193
	N	34	31	46	36	2.00E+02	7.94	63.00	3.46	1.261
4	60-1-No	45	52	41	46	2.50E-01	5.57	31.00	1.35	4.263
	N	33	32	38	34	2.00E+02	3.21	10.33	0.60	1.233
5	60-2-No	43	51	36	43	2.50E-01	7.51	56.33	2.63	4.235
	N	27	34	30	30	2.00E+02	3.51	12.33	0.82	1.179
6	60-3-No	41	43	38	41	2.50E-01	2.52	6.33	0.31	4.211
	N	26	32	35	31	2.00E+02	4.58	21.00	1.37	1.187
7	120-1-No	44	39	41	41	2.50E-01	2.52	6.33	0.31	4.218
	N	32	29	26	29	2.00E+02	3.00	9.00	0.62	1.160
8	120-2-No	40	35	46	40	2.50E-01	5.51	30.33	1.51	4.205
	N	27	29	30	29	2.00E+02	1.53	2.33	0.16	1.156
9	120-3-No	35	39	47	40	2.50E-01	6.11	37.33	1.87	4.204
	N	36	25	23	27	2.00E+02	7.00	49.00	3.57	1.138

Appendix 8.1(B)

COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^4.2 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No	39	43	44	42	2.50E-01	2.65	7.00	0.33	4.225
	N	39	33	41	38	2.00E+02	4.16	17.33	0.92	1.273
2	30-2-No	42	42	45	43	2.50E-01	1.73	3.00	0.14	4.235
	N	36	38	30	34	2.00E+02	4.16	17.33	1.01	1.237
3	30-3-No	50	38	43	43	2.50E-01	6.03	36.33	1.67	4.239
	N	39	34	33	35	2.00E+02	3.21	10.33	0.59	1.246
4	60-1-No	47	42	35	41	2.50E-01	6.03	36.33	1.77	4.215
	N	37	26	34	32	2.00E+02	5.69	32.33	2.02	1.204
5	60-2-No	33	43	47	41	2.50E-01	7.21	52.00	2.56	4.210
	N	37	32	23	30	2.00E+02	7.09	50.33	3.35	1.177
6	60-3-No	39	42	45	42	2.50E-01	3.00	9.00	0.43	4.225
	N	29	32	42	34	2.00E+02	6.81	46.33	2.73	1.229
7	120-1-No	36	41	47	41	2.50E-01	5.51	30.33	1.48	4.216
	N	30	24	23	25	2.00E+02	3.79	14.33	1.12	1.105
8	120-2-No	44	39	36	40	2.50E-01	4.04	16.33	0.83	4.199
	N	29	34	22	28	2.00E+02	6.03	36.33	2.61	1.144
9	120-3-No	43	47	40	43	2.50E-01	3.51	12.33	0.57	4.238
	N	27	22	36	28	2.00E+02	7.09	50.33	3.63	1.142

Appendix 8.2(A)

DOSE-RESPONSE COUNTS DATA IN COVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No-1	34	29	36	33	2.00E-04	3.61	13.00	0.79	7.216
	No-2	72	61	60	64	4.00E-04	6.66	44.33	1.38	7.205
	N-1	25	19	24	23	5.00E-01	3.21	10.33	0.92	3.653
	N-2	51	47	45	48	1.00E+00	3.06	9.33	0.39	3.678
2	30-2-No-1	28	30	34	31	2.00E-04	3.06	9.33	0.61	7.184
	No-2	74	60	62	65	4.00E-04	7.57	57.33	1.76	7.211
	N	54	46	42	47	1.00E+00	6.11	37.33	1.59	3.673
3	30-3-No-1	37	31	27	31	2.00E-04	5.03	25.33	1.61	7.196
	No-2	71	57	62	63	4.00E-04	7.09	50.33	1.60	7.198
	N-1	22	26	20	23	5.00E-01	3.06	9.33	0.83	3.654
	N-2	48	52	50	50	1.00E+00	2.00	4.00	0.16	3.699
4	30-4-No-1	39	32	30	33	2.00E-04	4.73	22.33	1.34	7.223
	No-2	71	59	65	65	4.00E-04	6.00	36.00	1.11	7.210
	N-1	27	22	20	23	5.00E-01	3.61	13.00	1.14	3.659
	N-2	51	53	54	53	1.00E+00	1.53	2.33	0.09	3.721
5	60-1-No-1	36	31	30	32	2.00E-04	3.21	10.33	0.64	7.207
	No-2	77	59	57	64	4.00E-04	11.02	121.33	3.81	7.202
	N	31	21	22	24	1.00E+00	5.51	30.33	2.50	3.385
6	60-2-No	74	62	57	64	4.00E-04	8.74	76.33	2.39	7.204
	N	42	28	39	36	2.00E+00	7.37	54.33	3.04	3.253

No.	Run Identification no.		Counts/plate		Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		1	2	3						
7	60-3-No	59	54	78	63	4.00E-04	12.66	160.33	5.10	7.196
	N-1	34	22	21	25	1.00E+00	7.23	52.33	4.18	3.399
	N-2	53	41	39	44	2.00E+00	7.57	57.33	2.61	3.342
8	120-1-No	68	53	71	63	4.00E-04	9.64	93.00	2.93	7,201
	N	46	61	54	53	5.00E+01	7.51	56.33	2.11	2.028
9	120-2-No	75	54	58	62	4.00E-04	11.15	124.33	4.03	7.188
	N-1	29	20	30	26	2.50E+01	5.51	30.33	2.34	2.016
	N-2	61	39	58	52	5.00E+01	11.93	142.33	5.51	2.014
10	120-3-No	78	59	56	64	4.00E-04	11.93	142.33	4.47	7.202
	N-1	26	34	21	26	2.50E+01	6.56	43.00	3.25	2.025
	N-2	47	59	61	55	5.00E+01	7.57	57.33	2.07	2.044

Appendix 8.2(B)

DOSE-RESPONSE COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^7.2 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No-1	27	35	35	32	2.00E-04	4.62	21.33	1.33	7.205
	No-2	69	65	70	68	4.00E-04	2.65	7.00	0.21	7.230
	N-1	27	20	22	23	5.00E-01	3.61	13.00	1.14	3.659
	N-2	47	46	53	49	1.00E+00	3.79	14.33	0.59	3.686
2	30-2-No-1	35	25	32	30	2.00E-04	5.13	26.33	1.73	7.181
	No-2	69	64	57	63	4.00E-04	6.03	36.33	1.15	7.198
	N-1	29	41	25	31	5.00E-01	8.33	69.33	4.48	3.792
	N-2	53	60	45	52	1.00E+00	7.51	56.33	2.15	3.719
3	30-3-No-1	29	37	35	33	2.00E-04	4.16	17.33	1.04	7.224
	No-2	72	49	59	59	4.00E-04	11.53	133.00	4.49	7.171
	N	49	55	48	51	1.00E+00	3.79	14.33	0.57	3.704
4	30-4-No-1	32	35	28	32	2.00E-04	3.51	12.33	0.78	7.198
	No-2	67	50	69	61	4.00E-04	10.44	109.00	3.55	7.186
	N-1	29	23	20	24	5.00E-01	4.58	21.00	1.77	3.676
	N-2	48	57	61	55	1.00E+00	6.66	44.33	1.61	3.741
5	60-1-No-1	34	29	32	32	2.00E-04	2.52	6.33	0.40	7.199
	No-2	69	56	64	63	4.00E-04	6.56	43.00	1.37	7.196
	N	36	24	20	26	1.00E+00	8.33	69.33	5.36	3.413
6	60-2-No	61	55	69	61	2.40E-04	7.02	49.33	1.61	7.408
	N	31	21	25	25	1.00E+00	5.03	25.33	2.00	3.404

No.	Run Identification no.	Counts/plate 1	Counts/plate 2	Counts/plate 3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
7	60-3-No	64	71	70	68	4.00E-04	3.79	14.33	0.42	7.232
	N-1	36	21	29	28	1.00E+00	7.51	56.33	4.03	3.447
	N-2	62	59	47	56	2.00E+00	7.94	63.00	2.27	3.444
8	120-1-No	49	69	66	61	4.00E-04	10.79	116.33	3.84	7.181
	N	49	58	65	57	5.00E+01	8.02	64.33	2.26	2.057
9	120-2-No	69	57	67	64	4.00E-04	6.43	41.33	1.29	7.205
	N	69	53	52	58	5.00E+01	9.54	91.00	3.16	2.061
10	120-3-No	76	51	69	64	4.00E-04	12.90	166.33	5.16	7.207
	N	31	20	34	28	2.50E+01	7.37	54.33	3.93	2.043

Appendix 8.3(A)

DOSE-RESPONSE COUNTS DATA IN COVERED SYSTEM, No ~ 10^9.3 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No	49	35	37	40	2.00E-06	7.57	57.33	2.87	9.300
	N	39	28	31	32	2.00E-06	5.69	32.33	2.00	9.209
2	30-2-No	47	43	36	42	2.00E-06	5.57	31.00	1.49	9.320
	N	35	37	31	34	2.00E-06	3.06	9.33	0.55	9.234
3	30-4-No	46	34	35	38	2.00E-06	6.66	44.33	2.34	9.278
	N-1	38	26	33	32	2.00E-06	6.03	36.33	2.27	9.203
	N-2	80	76	67	74	5.00E-06	6.66	44.33	1.20	9.171
4	30-5-No	34	45	37	38	2.00E-06	5.69	32.33	1.68	9.283
	N-1	41	25	29	31	2.00E-06	8.33	69.33	4.48	9.190
	N-2	68	76	77	74	5.00E-06	4.93	24.33	0.66	9.168
5	60-1-No	46	34	39	39	2.00E-06	6.03	36.33	1.85	9.294
	N	29	32	38	33	2.00E-06	4.58	21.00	1.28	9.215
6	60-2-No	39	42	35	39	2.00E-06	3.51	12.33	0.64	9.285
	N	42	25	29	31	2.00E-06	8.89	79.00	5.06	9.193
7	60-3-No	44	51	32	42	2.00E-06	9.61	92.33	4.44	9.318
	N	30	40	34	34	2.00E-06	5.03	25.33	1.47	9.236
8	60-4-No	34	39	42	38	2.00E-06	4.04	16.33	0.86	9.281
	N-1	32	29	31	31	2.00E-06	1.53	2.33	0.15	9.185
	N-2	64	78	73	71	5.00E-06	7.09	50.33	1.41	9.155

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D2	Log #/dL
9	120-1-No	34	43	39	38	2.00E-06	4.51	20.33	1.06	9.284
	N-1	32	27	27	29	2.00E-06	2.89	8.33	0.58	9.155
	N-2	78	74	67	73	5.00E-06	5.57	31.00	0.85	9.163
10	120-2-No	38	41	38	39	2.00E-06	1.73	3.00	0.15	9.290
	N-1	29	31	24	28	2.00E-06	3.61	13.00	0.93	9.144
	N-2	74	71	73	73	5.00E-06	1.53	2.33	0.06	9.162
11	120-3-No	38	35	41	38	2.00E-06	3.00	9.00	0.47	9.278
	N	28	33	22	27	2.00E-06	5.51	30.33	2.22	9.135

Appendix 8.3(B)

DOSE-RESPONSE COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^9.3 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D2	Log #/dL
		1	2	3						
1	30-1-No	39	33	42	38	2.00E-06	4.58	21.00	1.11	9.277
	N	28	39	30	32	2.00E-06	5.86	34.33	2.15	9.204
2	30-2-No	40	39	43	41	2.00E-06	2.08	4.33	0.21	9.308
	N	29	39	31	33	2.00E-06	5.29	28.00	1.71	9.214
3	30-3-No	45	39	31	38	2.00E-06	7.02	49.33	2.60	9.278
	N	28	40	26	31	2.00E-06	7.57	57.33	3.73	9.187
4	30-4-No	41	34	46	40	2.00E-06	6.03	36.33	1.82	9.301
	N	31	30	36	32	2.00E-06	3.21	10.33	0.64	9.207
5	60-1-No	42	49	31	40	2.00E-06	9.07	82.33	4.12	9.301
	N	34	28	36	32	2.00E-06	4.16	17.33	1.07	9.211
6	60-2-No	39	47	31	38	2.00E-06	8.00	64.00	3.33	9.284
	N	41	26	30	32	2.00E-06	7.77	60.33	3.80	9.201
7	60-3-No	43	39	36	39	2.00E-06	3.51	12.33	0.63	9.293
	N	29	33	32	31	2.00E-06	2.08	4.33	0.28	9.194
8	120-1-No	33	46	47	41	2.00E-06	7.81	61.00	2.94	9.317
	N-1	34	25	30	29	2.00E-06	4.51	20.33	1.38	9.168
	N-2	80	74	76	77	5.00E-06	3.06	9.33	0.24	9.185
9	120-2-No	39	47	33	39	2.00E-06	7.02	49.33	2.51	9.293
	N-1	26	28	33	29	2.00E-06	3.61	13.00	0.90	9.159
	N-2	75	76	68	73	5.00E-06	4.36	19.00	0.52	9.164

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D2	Log #/dL
		1	2	3						
10	120-3-No	33	42	41	38	2.00E-06	4.93	24.33	1.27	9.284
	N-1	23	37	27	28	2.00E-06	7.21	52.00	3.66	9.153
	N-2	74	73	77	75	5.00E-06	2.08	4.33	0.12	9.174

Appendix 8.4(A)

DOSE-RESPONSE COUNTS DATA IN COVERED SYSTEM, No ~ 10^11 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No	61	46	54	53	5.00E-08	7.51	56.33	2.11	11.028
	N	55	52	49	52	5.00E-08	3.00	9.00	0.35	11.017
2	30-2-No	45	62	53	53	5.00E-08	8.50	72.33	2.74	11.024
	N	58	51	46	51	5.00E-08	6.03	36.33	1.41	11.012
3	30-3-No	65	44	50	52	5.00E-08	10.82	117.00	4.47	11.019
	N	45	56	51	50	5.00E-08	5.51	30.33	1.20	11.004
4	30-4-No	47	65	54	55	5.00E-08	9.07	82.33	3.00	11.040
	N	59	58	45	54	5.00E-08	7.81	61.00	2.28	11.030
5	60-1-No	53	49	59	54	5.00E-08	5.03	25.33	0.95	11.029
	N	45	58	52	51	5.00E-08	6.51	42.33	1.65	11.012
6	60-2-No	59	48	51	52	5.00E-08	5.69	32.33	1.23	11.021
	N	56	43	53	50	5.00E-08	6.81	46.33	1.84	11.003
7	60-3-No	52	61	41	51	5.00E-08	10.02	100.33	3.96	11.006
	N	65	57	45	55	5.00E-08	10.07	101.33	3.68	11.042
8	60-4-No	51	52	59	54	5.00E-08	4.36	19.00	0.71	11.032
	N	45	51	60	52	5.00E-08	7.55	57.00	2.21	11.014

No.	Run Identification no.	Counts/plate			Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
		1	2	3						
9	120-1-No	48	58	57	54	5.00E-08	5.51	30.33	1.12	11.035
	N	47	49	59	51	5.00E-08	6.43	41.33	1.61	11.012
10	120-2-No	63	57	46	55	5.00E-08	8.62	74.33	2.71	11.040
	N	61	53	57	57	5.00E-08	4.00	16.00	0.56	11.056
11	120-3-No	56	58	45	53	5.00E-08	7.00	49.00	1.86	11.023
	N	48	46	61	51	5.00E-08	8.14	66.33	2.59	11.011
12	120-4-No	43	58	51	50	5.00E-08	7.51	56.33	2.24	11.003
	N	43	57	46	48	5.00E-08	7.37	54.33	2.25	10.985

Appendix 8.4(B)

DOSE-RESPONSE COUNTS DATA IN UNCOVERED SYSTEM, No ~ 10^11 CFU/dL, pH 6.9

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
1	30-1-No	42	65	51	52	5.00E-08	11.59	134.33	5.18	11.016
	N	59	52	43	51	5.00E-08	8.02	64.33	2.53	11.008
2	30-2-No	47	59	58	54	5.00E-08	6.66	44.33	1.63	11.036
	N	45	59	56	53	5.00E-08	7.37	54.33	2.05	11.025
3	30-3-No	63	46	55	54	5.00E-08	8.50	72.33	2.67	11.035
	N	58	53	57	56	5.00E-08	2.65	7.00	0.25	11.049
4	30-4-No	52	59	48	53	5.00E-08	5.57	31.00	1.17	11.024
	N	45	59	50	51	5.00E-08	7.09	50.33	1.97	11.009
5	60-1-No	61	52	62	58	5.00E-08	5.51	30.33	1.04	11.066
	N	53	62	54	56	5.00E-08	4.93	24.33	0.87	11.051
6	60-2-No	51	59	42	50	5.00E-08	8.50	72.33	2.88	11.002
	N	52	46	51	50	5.00E-08	3.21	10.33	0.42	10.996
7	60-3-No	56	52	47	52	5.00E-08	4.51	20.33	0.79	11.013
	N	48	55	48	50	5.00E-08	4.04	16.33	0.65	11.002
8	60-4-No	43	57	56	52	5.00E-08	7.81	61.00	2.37	11.014
		53	45	51	50	5.00E-08	4.16	17.33	0.70	10.996

No.	Run Identification no.	Counts/plate 1	2	3	Geometric mean	Vol. filtered (mL)	Std. Dev.	Variance	D^2	Log #/dL
9	120-1-No	58	60	43	53	5.00E-08	9.29	86.33	3.25	11.026
	N	55	52	46	51	5.00E-08	4.58	21.00	0.83	11.007
10	120-2-No	58	43	57	52	5.00E-08	8.39	70.33	2.70	11.019
	N	49	51	53	51	5.00E-08	2.00	4.00	0.16	11.008
11	120-3-No	58	64	48	56	5.00E-08	8.08	65.33	2.32	11.051
	N	56	56	53	55	5.00E-08	1.73	3.00	0.11	11.041
12	120-4-No	67	56	57	60	5.00E-08	6.08	37.00	1.24	11.078
	N	59	61	54	58	5.00E-08	3.61	13.00	0.45	11.064

Appendix 9.1(A)

SUMMARY: DOSE-RESPONSE, No ~ 10^4.2 CFU/dL, pH 6.9

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (=No-N)
1	30	C	46	8	1.78E+04	1.87E+01	1.05E-03	-2.98	1.78E+04
		U	46	8	1.68E+04	1.87E+01	1.12E-03	-2.95	1.68E+04
2	30	C	46	8	1.66E+04	1.75E+01	1.05E-03	-2.98	1.66E+04
		U	46	8	1.72E+04	1.73E+01	1.00E-03	-3.00	1.72E+04
3	30	C	46	8	1.56E+04	1.82E+01	1.17E-03	-2.93	1.56E+04
		U	46	8	1.73E+04	1.76E+01	1.02E-03	-2.99	1.73E+04
4	60	C	46	8	1.83E+04	1.71E+01	9.33E-04	-3.03	1.83E+04
		U	46	8	1.64E+04	1.60E+01	9.75E-04	-3.01	1.64E+04
5	60	C	46	8	1.72E+04	1.51E+01	8.79E-04	-3.06	1.72E+04
		U	46	8	1.62E+04	1.50E+01	9.27E-04	-3.03	1.62E+04
6	60	C	46	8	1.63E+04	1.54E+01	9.46E-04	-3.02	1.62E+04
		U	46	8	1.68E+04	1.69E+01	1.01E-03	-3.00	1.68E+04
7	120	C	46	8	1.65E+04	1.45E+01	8.75E-04	-3.06	1.65E+04
		U	47	9	1.64E+04	1.27E+01	7.74E-04	-3.11	1.64E+04
8	120	C	46	8	1.60E+04	1.43E+01	8.93E-04	-3.05	1.60E+04
		U	47	8	1.58E+04	1.39E+01	8.81E-04	-3.06	1.58E+04
9	120	C	47	8	1.60E+04	1.37E+01	8.59E-04	-3.07	1.60E+04
		U	47	9	1.73E+04	1.39E+01	8.02E-04	-3.10	1.73E+04

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	5E+11	0	8	5E+11
	6E+11	0	7	6E+11
2	6E+11	0	7	6E+11
	6E+11	0	7	5E+11
3	6E+11	0	7	6E+11
	6E+11	0	8	6E+11
4	6E+11	0	8	5E+11
	6E+11	0	8	6E+11
5	6E+11	0	8	6E+11
	6E+11	0	8	6E+11
6	6E+11	0	8	6E+11
	6E+11	0	8	6E+11
7	6E+11	0	8	6E+11
	7E+11	1	8	6E+11
8	7E+11	0	8	6E+11
	7E+11	1	8	6E+11
9	7E+11	0	8	6E+11
	6E+11	1	8	6E+11

Appendix 9.1(B)

SUMMARY: DOSE-RESPONSE, No ~ 10^4.2 CFU/dL, pH 6.9

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (=No-N)
1	30	C	45.84	7.75	1.78E+04	1.87E+01	1.05E-03	-2.98	1.78E+04
		U	45.72	7.63	1.68E+04	1.87E+01	1.12E-03	-2.95	1.68E+04
2	30	C	45.60	7.51	1.66E+04	1.75E+01	1.05E-03	-2.98	1.66E+04
		U	45.72	7.63	1.72E+04	1.73E+01	1.00E-03	-3.00	1.72E+04
3	30	C	45.60	7.51	1.56E+04	1.82E+01	1.17E-03	-2.93	1.56E+04
		U	45.96	7.87	1.73E+04	1.76E+01	1.02E-03	-2.99	1.73E+04
4	60	C	46.20	8.11	1.83E+04	1.71E+01	9.33E-04	-3.03	1.83E+04
		U	46.20	8.11	1.64E+04	1.60E+01	9.75E-04	-3.01	1.64E+04
5	60	C	46.20	8.11	1.72E+04	1.51E+01	8.79E-04	-3.06	1.72E+04
		U	46.42	8.33	1.62E+04	1.50E+01	9.27E-04	-3.03	1.62E+04
6	60	C	46.08	7.99	1.63E+04	1.54E+01	9.46E-04	-3.02	1.62E+04
		U	46.30	8.21	1.68E+04	1.69E+01	1.01E-03	-3.00	1.68E+04
7	120	C	46.30	8.21	1.65E+04	1.45E+01	8.75E-04	-3.06	1.65E+04
		U	46.66	8.57	1.64E+04	1.27E+01	7.74E-04	-3.11	1.64E+04
8	120	C	46.42	8.33	1.60E+04	1.43E+01	8.93E-04	-3.05	1.60E+04
		U	46.54	8.45	1.58E+04	1.39E+01	8.81E-04	-3.06	1.58E+04
9	120	C	46.54	8.45	1.60E+04	1.37E+01	8.59E-04	-3.07	1.60E+04
		U	46.67	8.58	1.73E+04	1.39E+01	8.02E-04	-3.10	1.73E+04

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	5.45E+11	0.11	7.64	5.37E+11
	5.70E+11	0.20	7.43	5.54E+11
2	5.68E+11	0.11	7.40	5.58E+11
	5.57E+11	0.20	7.43	5.41E+11
3	6.04E+11	0.11	7.40	5.94E+11
	5.69E+11	0.20	7.67	5.53E+11
4	5.55E+11	0.21	7.90	5.39E+11
	6.20E+11	0.34	7.77	5.93E+11
5	5.92E+11	0.21	7.90	5.75E+11
	6.44E+11	0.35	7.98	6.16E+11
6	6.17E+11	0.21	7.78	5.99E+11
	6.13E+11	0.34	7.87	5.86E+11
7	6.23E+11	0.39	7.82	5.92E+11
	6.54E+11	0.62	7.95	6.05E+11
8	6.52E+11	0.40	7.93	6.19E+11
	6.70E+11	0.61	7.84	6.21E+11
9	6.63E+11	0.41	8.04	6.29E+11
	6.22E+11	0.62	7.96	5.76E+11

Appendix 9.2(A)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2/dL, pH 6.9

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30	C	46	11	1.63E+07	4.63E+03	2.85E-04	-3.55	1.63E+07
		U	46	11	1.51E+07	4.71E+03	3.11E-04	-3.51	1.51E+07
2	30	C	46	10	1.58E+07	4.71E+03	2.99E-04	-3.53	1.58E+07
		U	46	10	1.55E+07	5.70E+03	3.68E-04	-3.43	1.55E+07
3	30	C	46	10	1.57E+07	4.75E+03	3.02E-04	-3.52	1.57E+07
		U	46	10	1.58E+07	5.06E+03	3.21E-04	-3.49	1.58E+07
4	30	C	46	11	1.65E+07	4.90E+03	2.97E-04	-3.53	1.65E+07
		U	46	11	1.56E+07	5.12E+03	3.29E-04	-3.48	1.56E+07
5	60	C	47	11	1.60E+07	2.43E+03	1.51E-04	-3.82	1.60E+07
		U	47	11	1.58E+07	2.59E+03	1.64E-04	-3.79	1.58E+07
6	60	C	46	11	1.60E+07	1.79E+03	1.12E-04	-3.95	1.60E+07
		U	46	12	2.56E+07	2.54E+03	9.91E-05	-4.00	2.56E+07
7	60	C	45	12	1.57E+07	2.35E+03	1.50E-04	-3.83	1.57E+07
		U	45	12	1.71E+07	2.79E+03	1.64E-04	-3.79	1.71E+07
8	120	C	46	13	1.59E+07	1.07E+02	6.71E-06	-5.17	1.59E+07
		U	46	14	1.52E+07	1.14E+02	7.52E-06	-5.12	1.52E+07
9	120	C	46	12	1.54E+07	1.04E+02	6.71E-06	-5.17	1.54E+07
		U	46	12	1.60E+07	1.15E+02	7.18E-06	-5.14	1.60E+07
10	120	C	45	13	1.59E+07	1.08E+02	6.81E-06	-5.17	1.59E+07
		U	45	13	1.61E+07	1.10E+02	6.85E-06	-5.16	1.61E+07

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	8E+08	0	10	8E+08
	9E+08	0	10	9E+08
2	8E+08	0	10	8E+08
	8E+08	0	10	8E+08
3	8E+08	0	10	8E+08
	8E+08	0	10	8E+08
4	8E+08	0	11	8E+08
	9E+08	0	10	8E+08
5	9E+08	0	11	8E+08
	9E+08	0	11	8E+08
6	9E+08	0	11	8E+08
	6E+08	0	12	6E+08
7	1E+09	0	12	9E+08
	9E+08	1	12	8E+08
8	1E+09	1	12	9E+08
	1E+09	1	13	1E+09
9	1E+09	1	12	9E+08
	1E+09	1	11	9E+08
10	1E+09	1	12	1E+09
	1E+09	1	12	1E+09

Appendix 9.2(B)

SUMMARY: DOSE-RESPONSE, No ~ 10^7.2/dL, pH 6.9

Run no.	Time (sec)	System	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30	C	46.32	10.61	1.63E+07	4.63E+03	2.85E-04	-3.55	1.63E+07
		U	46.32	10.61	1.51E+07	4.71E+03	3.11E-04	-3.51	1.51E+07
2	30	C	45.96	10.25	1.58E+07	4.71E+03	2.99E-04	-3.53	1.58E+07
		U	45.96	10.25	1.55E+07	5.70E+03	3.68E-04	-3.43	1.55E+07
3	30	C	46.20	10.49	1.57E+07	4.75E+03	3.02E-04	-3.52	1.57E+07
		U	46.20	10.49	1.58E+07	5.06E+03	3.21E-04	-3.49	1.58E+07
4	30	C	46.42	10.71	1.65E+07	4.90E+03	2.97E-04	-3.53	1.65E+07
		U	46.42	10.71	1.56E+07	5.12E+03	3.29E-04	-3.48	1.56E+07
5	60	C	46.78	11.07	1.60E+07	2.43E+03	1.51E-04	-3.82	1.60E+07
		U	46.78	11.07	1.58E+07	2.59E+03	1.64E-04	-3.79	1.58E+07
6	60	C	45.72	11.01	1.60E+07	1.79E+03	1.12E-04	-3.95	1.60E+07
		U	45.60	12.27	2.56E+07	2.54E+03	9.91E-05	-4.00	2.56E+07
7	60	C	45.48	12.15	1.57E+07	2.35E+03	1.50E-04	-3.83	1.57E+07
		U	45.36	12.03	1.71E+07	2.79E+03	1.64E-04	-3.79	1.71E+07
8	120	C	45.96	12.63	1.59E+07	1.07E+02	6.71E-06	-5.17	1.59E+07
		U	45.96	13.81	1.52E+07	1.14E+02	7.52E-06	-5.12	1.52E+07
9	120	C	45.90	12.27	1.54E+07	1.04E+02	6.71E-06	-5.17	1.54E+07
		U	45.90	12.39	1.60E+07	1.15E+02	7.18E-06	-5.14	1.60E+07
10	120	C	45.24	12.87	1.59E+07	1.08E+02	6.81E-06	-5.17	1.59E+07
		U	45.24	13.33	1.61E+07	1.10E+02	6.85E-06	-5.16	1.61E+07

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	8.18E+08	0.15	10.46	8.05E+08
	8.79E+08	0.28	10.33	8.54E+08
2	8.14E+08	0.14	10.11	8.01E+08
	8.30E+08	0.27	9.98	8.06E+08
3	8.35E+08	0.15	10.34	8.22E+08
	8.34E+08	0.27	10.22	8.10E+08
4	8.15E+08	0.15	10.56	8.01E+08
	8.63E+08	0.28	10.43	8.38E+08
5	8.65E+08	0.29	10.78	8.41E+08
	8.79E+08	0.46	10.61	8.41E+08
6	8.63E+08	0.29	10.72	8.38E+08
	6.01E+08	0.46	11.81	5.77E+08
7	9.70E+08	0.32	11.83	9.42E+08
	8.84E+08	0.51	11.52	8.45E+08
8	9.96E+08	0.61	12.02	9.46E+08
	1.14E+09	0.99	12.82	1.06E+09
9	9.97E+08	0.59	11.68	9.47E+08
	9.68E+08	0.89	11.50	8.97E+08
10	1.01E+09	0.62	12.25	9.62E+08
	1.04E+09	0.96	12.37	9.60E+08

Appendix 9.3(A-1)

SUMMARY: DOSE-RESPONSE IN COVERED SYSTEM, No ~ 10^ 9.3 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30	46	18	2.00E+09	1.62E+09	0.81	-0.09	3.77E+08
2	30	45	17	2.09E+09	1.71E+09	0.82	-0.09	3.75E+08
3	30	45	17	1.90E+09	1.54E+09	0.81	-0.09	3.59E+08
4	30	45	17	1.92E+09	1.51E+09	0.79	-0.10	4.09E+08
5	60	47	23	1.97E+09	1.64E+09	0.83	-0.08	3.27E+08
6	60	46	22	1.93E+09	1.56E+09	0.81	-0.09	3.68E+08
7	60	45	22	2.08E+09	1.72E+09	0.83	-0.08	3.58E+08
8	60	46	22	1.91E+09	1.48E+09	0.77	-0.11	4.31E+08
9	120	46	41	1.92E+09	1.44E+09	0.75	-0.13	4.81E+08
10	120	46	41	1.95E+09	1.42E+09	0.73	-0.14	5.28E+08
11	120	46	41	1.90E+09	1.36E+09	0.72	-0.14	5.32E+08

Run no.	Total O3 molecules utilized per bacteria removal	Ozone decom. during reaction (ug/L), using equations	Ozone used in disinfecting E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	6E+07	0	17	6E+07
2	6E+07	0	16	5E+07
3	6E+07	0	16	6E+07
4	5E+07	0	17	5E+07
5	9E+07	1	22	8E+07
6	8E+07	1	21	7E+07
7	8E+07	1	21	7E+07
8	6E+07	1	21	6E+07
9	1E+08	2	39	1E+08
10	1E+08	2	39	9E+07
11	1E+08	2	39	9E+07

Appendix 9.3(B-1)

SUMMARY: DOSE-RESPONSE IN COVERED SYSTEM, No ~ 10^ 9.3 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30	46.20	17.63	2.00E+09	1.62E+09	0.81	-0.09	3.77E+08
2	30	45.24	16.67	2.09E+09	1.71E+09	0.82	-0.09	3.75E+08
3	30	45.24	16.67	1.90E+09	1.54E+09	0.81	-0.09	3.59E+08
4	30	45.48	16.91	1.92E+09	1.51E+09	0.79	-0.10	4.09E+08
5	60	46.54	22.73	1.97E+09	1.64E+09	0.83	-0.08	3.27E+08
6	60	45.84	22.03	1.93E+09	1.56E+09	0.81	-0.09	3.68E+08
7	60	45.48	21.67	2.08E+09	1.72E+09	0.83	-0.08	3.58E+08
8	60	46.08	22.27	1.91E+09	1.48E+09	0.77	-0.11	4.31E+08
9	120	45.96	41.20	1.92E+09	1.44E+09	0.75	-0.13	4.81E+08
10	120	45.60	40.84	1.95E+09	1.42E+09	0.73	-0.14	5.28E+08
11	120	45.96	41.20	1.90E+09	1.36E+09	0.72	-0.14	5.32E+08

Run no.	Total O3 molecules utilized per bacteria removal	Ozone decom. during reaction (ug/L), using equations	Ozone used in disinfecting E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	5.86E+07	0.25	17.38	5.76E+07
2	5.57E+07	0.23	16.44	5.48E+07
3	5.83E+07	0.23	16.44	5.73E+07
4	5.19E+07	0.24	16.67	5.10E+07
5	8.70E+07	0.57	22.16	8.46E+07
6	7.50E+07	0.56	21.47	7.29E+07
7	7.59E+07	0.58	21.09	7.37E+07
8	6.48E+07	1.07	21.20	6.15E+07
9	1.07E+08	1.98	39.22	1.02E+08
10	9.70E+07	1.96	38.88	9.21E+07
11	9.70E+07	1.98	39.22	9.22E+07

Appendix 9.3(A-2)

SUMMARY: DOSE-RESPONSE IN UNCOVERED SYSTEM, No ~ 10^9.3 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (=No-N)
1	30	45	17	1.89E+09	1.60E+09	0.85	-0.07	2.93E+08
2	30	46	18	2.03E+09	1.64E+09	0.81	-0.09	3.96E+08
3	30	46	18	1.90E+09	1.54E+09	0.81	-0.09	3.59E+08
4	30	45	17	2.00E+09	1.61E+09	0.81	-0.09	3.89E+08
5	60	47	23	2.00E+09	1.63E+09	0.81	-0.09	3.74E+08
6	60	47	23	1.92E+09	1.59E+09	0.83	-0.08	3.35E+08
7	60	47	23	1.96E+09	1.56E+09	0.80	-0.10	4.00E+08
8	120	46	41	2.07E+09	1.50E+09	0.72	-0.14	5.72E+08
9	120	46	42	1.96E+09	1.45E+09	0.74	-0.13	5.11E+08
10	120	45	40	1.92E+09	1.46E+09	0.76	-0.12	4.64E+08

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting E.coli	Actual ozone molecules used per bacterium removal
1	7E+07	0	16	7E+07
2	6E+07	0	17	5E+07
3	6E+07	0	17	6E+07
4	5E+07	0	16	5E+07
5	8E+07	1	22	7E+07
6	9E+07	1	22	8E+07
7	7E+07	1	22	7E+07
8	9E+07	3	38	8E+07
9	1E+08	3	39	9E+07
10	1E+08	3	38	1E+08

Appendix 9.3(B-2)

SUMMARY: DOSE-RESPONSE IN UNCOVERED SYSTEM, No ~ 10^9.3 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (=No-N)
1	30	45.48	16.91	1.89E+09	1.60E+09	0.85	-0.07	2.93E+08
2	30	46.20	17.63	2.03E+09	1.64E+09	0.81	-0.09	3.96E+08
3	30	46.08	17.51	1.90E+09	1.54E+09	0.81	-0.09	3.59E+08
4	30	45.48	16.91	2.00E+09	1.61E+09	0.81	-0.09	3.89E+08
5	60	46.78	22.97	2.00E+09	1.63E+09	0.81	-0.09	3.74E+08
6	60	46.54	22.73	1.92E+09	1.59E+09	0.83	-0.08	3.35E+08
7	60	46.66	22.85	1.96E+09	1.56E+09	0.80	-0.10	4.00E+08
8	120	46.20	41.44	2.07E+09	1.50E+09	0.72	-0.14	5.72E+08
9	120	46.30	41.54	1.96E+09	1.45E+09	0.74	-0.13	5.11E+08
10	120	45.24	40.48	1.92E+09	1.46E+09	0.76	-0.12	4.64E+08

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone Used in disinfecting E.coli	Actual ozone molecules used per bacterium removal
1	7.24E+07	0.44	16.47	7.03E+07
2	5.59E+07	0.46	17.17	5.43E+07
3	6.12E+07	0.46	17.05	5.95E+07
4	5.44E+07	0.44	16.47	5.29E+07
5	7.69E+07	0.96	22.01	7.35E+07
6	8.51E+07	0.95	21.78	8.14E+07
7	7.15E+07	0.96	21.89	6.84E+07
8	9.08E+07	2.98	38.46	8.41E+07
9	1.02E+08	2.99	38.55	9.43E+07
10	1.09E+08	2.91	37.57	1.01E+08

Appendix 9.4(A-1)

SUMMARY: DOSE-RESPONSE IN COVERED SYSTEM, No ~ 10^ 11 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30	46	36	1.067E+11	1.040E+11	0.97	-0.011	2.67E+09
2	30	45	36	1.057E+11	1.028E+11	0.97	-0.012	2.88E+09
3	30	46	37	1.045E+11	1.009E+11	0.97	-0.015	3.55E+09
4	30	46	36	1.096E+11	1.072E+11	0.98	-0.010	2.50E+09
5	60	45	41	1.069E+11	1.028E+11	0.96	-0.017	4.10E+09
6	60	46	41	1.050E+11	1.007E+11	0.96	-0.018	4.26E+09
8	60	46	41	1.076E+11	1.033E+11	0.96	-0.018	4.37E+09
9	120	46	46	1.084E+11	1.028E+11	0.95	-0.023	5.59E+09
11	120	46	46	1.054E+11	1.026E+11	0.97	-0.012	2.87E+09
12	120	46	46	1.007E+11	9.661E+10	0.96	-0.018	4.09E+09

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	2E+07	1	36	2E+07
2	2E+07	1	35	2E+07
3	1E+07	1	36	1E+07
4	2E+07	1	36	2E+07
5	1E+07	1	40	1E+07
6	1E+07	1	40	1E+07
8	1E+07	1	40	1E+07
9	1E+07	2	44	1E+07
11	2E+07	2	44	2E+07
12	1E+07	2	44	1E+07

Appendix 9.4(A-2)

SUMMARY: DOSE-RESPONSE IN UNCOVERED SYSTEM, No ~ 10^11 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	Log No/dL	Log (N/No)	Removal/dL (=No-N)
1	30	44	35	1.038E+11	1.019E+11	11.02	-0.008	1.89E+09
2	30	45	36	1.086E+11	1.059E+11	11.04	-0.011	2.72E+09
4	30	46	37	1.057E+11	1.021E+11	11.02	-0.015	3.59E+09
5	60	46	41	1.164E+11	1.125E+11	11.07	-0.015	3.95E+09
6	60	46	41	1.005E+11	9.908E+10	11.00	-0.006	1.38E+09
7	60	45	41	1.030E+11	1.005E+11	11.01	-0.011	2.58E+09
8	60	46	41	1.033E+11	9.908E+10	11.01	-0.018	4.19E+09
9	120	46	46	1.062E+11	1.016E+11	11.03	-0.019	4.54E+09
10	120	45	45	1.045E+11	1.019E+11	11.02	-0.011	2.61E+09
11	120	45	45	1.125E+11	1.099E+11	11.05	-0.010	2.56E+09
12	120	46	46	1.197E+11	1.159E+11	11.08	-0.014	3.80E+09

Run no.	Total O3 molecules utilized per bacteria removal	Ozone decom. during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	2E+07	1	34	2E+07
2	2E+07	1	35	2E+07
4	1E+07	1	36	1E+07
5	1E+07	2	39	1E+07
6	4E+07	2	40	4E+07
7	2E+07	2	39	2E+07
8	1E+07	2	40	1E+07
9	1E+07	2	44	1E+07
10	2E+07	2	44	2E+07
11	2E+07	2	43	2E+07
12	2E+07	2	44	1E+07

Appendix 9.4(B-1)

SUMMARY: DOSE-RESPONSE IN COVERED SYSTEM, No ~ 10^ 11 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	N/No	Log (N/No)	Removal/dL (= No-N)
1	30.00	45.95	36	1.067E+11	1.040E+11	0.97	-0.011	2.67E+09
2	30.00	45.48	36	1.057E+11	1.028E+11	0.97	-0.012	2.88E+09
3	30.00	46.19	37	1.045E+11	1.009E+11	0.97	-0.015	3.55E+09
4	30.00	45.95	36	1.096E+11	1.072E+11	0.98	-0.010	2.50E+09
5	60.00	45.48	41	1.069E+11	1.028E+11	0.96	-0.017	4.10E+09
6	60.00	45.95	41	1.050E+11	1.007E+11	0.96	-0.018	4.26E+09
8	60.00	45.95	41	1.076E+11	1.033E+11	0.96	-0.018	4.37E+09
9	120.00	46.19	46	1.084E+11	1.028E+11	0.95	-0.023	5.59E+09
11	120.00	46.19	46	1.054E+11	1.026E+11	0.97	-0.012	2.87E+09
12	120.00	46.43	46	1.007E+11	9.661E+10	0.96	-0.018	4.09E+09

Run no.	Total O3 molecules utilized per bacterium removal	Ozone decomposed during reaction (ug/L), using equations	Ozone used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	1.71E+07	0.51	35.92	1.68E+07
2	1.56E+07	0.50	35.46	1.54E+07
3	1.30E+07	0.51	36.16	1.27E+07
4	1.83E+07	0.51	35.92	1.80E+07
5	1.24E+07	1.06	39.66	1.21E+07
6	1.21E+07	1.07	40.12	1.18E+07
8	1.18E+07	1.07	40.12	1.15E+07
9	1.04E+07	2.22	43.97	9.83E+06
11	2.01E+07	2.22	43.97	1.91E+07
12	1.42E+07	2.23	44.20	1.35E+07

Appendix 9.4(B-2)

SUMMARY: DOSE-RESPONSE IN UNCOVERED SYSTEM, No ~ 10^11 CFU/dL, pH 6.9

Run no.	Time (sec)	Ozone dose applied (ug/L)	Ozone utilized (ug/L)	No/dL	N/dL	Log No/dL	Log (N/No)	Removal/dL (=No-N)
1	30	44.29	34.77	1.038E+11	1.019E+11	11.02	-0.008	1.89E+09
2	30	45.48	35.96	1.086E+11	1.059E+11	11.04	-0.011	2.72E+09
4	30	46.43	36.91	1.057E+11	1.021E+11	11.02	-0.015	3.59E+09
5	60	45.71	40.95	1.164E+11	1.125E+11	11.07	-0.015	3.95E+09
6	60	46.19	41.43	1.005E+11	9.908E+10	11.00	-0.006	1.38E+09
7	60	45.48	40.72	1.030E+11	1.005E+11	11.01	-0.011	2.58E+09
8	60	46.19	41.43	1.033E+11	9.908E+10	11.01	-0.018	4.19E+09
9	120	46.19	46.19	1.062E+11	1.016E+11	11.03	-0.019	4.54E+09
10	120	45.48	45.48	1.045E+11	1.019E+11	11.02	-0.011	2.61E+09
11	120	45.00	45.00	1.125E+11	1.099E+11	11.05	-0.010	2.56E+09
12	120	45.95	45.95	1.197E+11	1.159E+11	11.08	-0.014	3.80E+09

Run no.	Total O3 molecules utilized per bacteria removal	Ozone decom. during reaction (ug/L), using equations	Ozone Used in disinfecting the E.coli (ug/L)	Actual ozone molecules used per bacterium removal
1	2.30E+07	0.90	33.87	2.24E+07
2	1.66E+07	0.93	35.03	1.61E+07
4	1.29E+07	0.96	35.95	1.25E+07
5	1.30E+07	1.72	39.23	1.24E+07
6	3.77E+07	1.74	39.69	3.60E+07
7	1.98E+07	1.71	39.01	1.89E+07
8	1.24E+07	1.74	39.69	1.18E+07
9	1.27E+07	1.94	44.25	1.22E+07
10	2.18E+07	1.91	43.57	2.08E+07
11	2.20E+07	1.89	43.11	2.11E+07
12	1.52E+07	1.93	44.02	1.45E+07